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DERIVATIVES FROM AN UNUSUAL STRAIN OF TOBACCO-MOSAIC VIRUS

I. P. NORVAL[1]

(Accepted for publication July 21, 1938)

In recent years it has been recognized (3, 5, 6, 7, 9, 12) that the plant viruses often give rise spontaneously to new forms. These forms or strains usually preserve their identity in succeeding host passages and, like the parent virus, occasionally mutate to give still other strains, which can be distinguished from them.

Jensen (7) obtained from the common tobacco mosaic an unusual variant, J–14[2], which is only slightly infectious in Turkish tobacco, *Nicotiana tabacum* L. and tomato, *Lycopersicum esculentum* Mill., causes spreading necrotic lesions, and never becomes systemic. It seemed likely that a study of a large number of variants from strain J–14 might be of value in enabling us to understand more about the "genetics" of the tobacco-mosaic virus group. The purpose of this paper is to present the results of such a study.

MATERIALS AND METHODS

Since the isolation of strain J–14 by Jensen, the virus had been maintained by serial passage in tobacco. Inoculations from a stem lesion were made to the leaves of healthy tobacco plants. A few necrotic primary lesions resulted. Three serial transfers were made to tobacco from single necrotic lesions of this stock. Plants of the first passage were held for about 20 days and in no case showed signs of a second virus. This was considered sufficient evidence that the virus was free from contamination.

It is so difficult to obtain infection with J–14 that carborundum (No. 320) was always either dusted on the leaves or added directly to the inoculum. For transferring from single lesions on leaves Holmes' (4) brass-tube method was employed. At the beginning of the investigation (Table 1) 2–4 plants usually were inoculated from a single lesion. Later (Tables 2, 3, and 4) it was found more convenient to crush several leaf lesions, or a single stem lesion, in a mortar and to inoculate from this with a cloth-covered glass spatula. In this way more uniform infection could be obtained with an average of about 3 lesions per plant. The hand which supported the leaf to be inoculated was covered with a piece of waxed paper. Instruments were sterilized by boiling or autoclaving.

Since J–14 spreads slowly, it was presumed that almost all mutants arising from it would be faster-moving and would probably be able to cause systemic infections on the upper leaves of the plants. From these leaves,

[1] Commonwealth Fund Fellow.

[2] The initials J, K and N have been used to denote strains of the virus isolated by Jensen, Kunkel and the writer, respectively.

TABLE 1.—*Variants isolated from tobacco-mosaic virus, strain J–14, in Turkish tobacco plants infected with virus from single lesions*

Source of inoculum	No. of plants infected	Mutations		Days to appearance
		Strain number	Type in Turkish tobacco	
L[a]	14	.....		.....
L	12	1	Local, yellow	6
L	13	.....		.....
S	8	.....		.....
L	6	14	Local, yellow	5
L	9	18	Local, yellow	.....
		19	Local, yellow	.....
L	63	2	Systemic, necrotic	10
		4	Fast-moving, yellow	8
		5	Fast-moving, yellow-green	10
S	12	7	Fast-moving, yellow	10
		6	Distorting, green	.....
L	65	12	Fast-moving, yellow	10
		13	Local, yellow	10
		10	Non-distorting, green	10
		15	Non-distorting, green	20 ±
		17	Distorting, green	20 ±

[a] L indicates inoculum was from a leaf lesion; S that it was from a stem lesion in a Turkish tobacco plant.

TABLE 2.—*Variants isolated from tobacco-mosaic virus, strain J–14, in Turkish tobacco plants infected with virus from several different local lesions*

Source of inoculum	No. of plants infected	Mutations		Days to appearance
		Strain number	Type in Turkish tobacco	
S	6	.....		.....
L	9	.....		.....
L	32	41	Severe, yellow	12 ±
		46	Severe, yellow	.....
		44	Fast-moving, yellow	10
		53	Distorting, green	30
L	39	49	Fast-moving, yellow	6
		50	Slow-moving, yellow	10
L	24	52	Fast-moving, yellow	9
L	3	55	Distorting, green	8
L	23	59	Distorting, green	32
		60	Distorting, green	34
L	35	57	Local, yellow	7
		58	Distorting, green	17
S	15	.....		.....
S	6	.....		.....
L	23	68	Fast-moving, yellow	6
		82	Non-distorting, green	6
L	20	94	Distorting, green	36
S	10	87	Non-distorting, green	15
		88	Slow-moving, yellow	.....

TABLE 3.—*Variants obtained from old stem lesions in Turkish tobacco*

Date	Source of inoculum	No. of plants infected	Mutations		Days to appearance
			Strain number	Type in Turkish tobacco	
Feb. 11, 1938	S	15	90	Distorting, green	5
			92	Local, yellow	7
			93	Non-distorting, green	7
			97	Fast-moving, yellow	12
Feb. 14, "	S	10	96	Fast-moving, yellow	5
			98	Fast-moving, yellow	7
			99	Distorting, green	9
Feb. 15, "	S	15	100	Severe, yellow	8
			102	Distorting, green	13
			103	Slow-moving, yellow	10
Mar. 8, "	S	15	106	Severe, yellow	8
			107	Slow-moving, yellow	7
			126	Fast-moving, yellow	8
			108	Distorting, green	5
			112	Severe, yellow	7
			114	Severe, yellow	10
Mar. 9, "	S	20	115	Distorting, green	6
			111	Severe, yellow	6
			116	Fast-moving, yellow	10
			117	Distorting, green	7
			118	Local, yellow	6
Mar. 10, "	S	20	119	Fast-moving, yellow	6
			120	Local, yellow	6
			121	Fast-moving, yellow	8
			122	Local, yellow	8
			123	Slow-moving, yellow	9
			124	Severe, yellow	10
			125	Distorting, green	15

TABLE 4.—*Variants isolated from tobacco-mosaic virus, strain J–14, in tomato and N. sylvestris*

Source of inoculum	No. of plants infected	Mutations		Days to appearance
		Strain number	Type in Turkish tobacco	
L from tobacco	2			
S from tomato	6			
L from tobacco	15			
L from tomato	14			
S from tobacco	6	62	Fast-moving, yellow	24
		80	Systemic, necrotic	16
S from tomato	40	69	Fast-moving, yellow	12
		79	Distorting, green	15
		63	Fast-moving, yellow	8
Nicotiana sylvestris				
L from tobacco	34	83	Local, yellow	7
		84	Non-distorting, green	9
L from *N. sylvestris*	10 (tobacco)	95	Local, yellow	6
		101	Non-distorting, green	10

it should be possible to remove them uncontaminated with the parent strain. This method was employed throughout the investigation for obtaining variants. Rarely did more than one variant appear in the same plant. However, in view of the possibility that more than one might arise, the mutants were always taken first to *Nicotiana glutinosa* L. and thence by single-lesion inoculation back to tobacco (5).

Mutations arising at the edge of a necrotic lesion might conceivably be unable to move away from the lesion but might be segregated if inoculations were made from the crushed-up lesion. If such a mutant were present in considerable quantity it might appear in all the inoculated plants. Instead of using large sets of plants infected from one source of inoculum, many small sets of plants were, therefore, used and frequent transfers were made from set to set. When, as often happened, a number of systemic strains closely resembling each other appeared in the same set, no attempt was made to distinguish between them, the presumption being that they arose in the parent lesion as a single mutation and did not move away. Each variant was assigned a number when it was first seen. Subsequently, if several variants arose in the same set and appeared to be identical, only one was retained. For this reason the numerals designating the strains in the text are higher than the number of strains isolated.

Except where otherwise stated, the experiments were carried out during the autumn and winter months of 1936 and 1937 in a greenhouse held at about 75° F. and kept comparatively free from insects by regular fumigation.

RESULTS

Sixty-two variants were isolated from strain J–14 by passage to Turkish tobacco, tomato and *Nicotiana sylvestris* Speg. and Comes plants. The data concerning their isolation are summarised in tables 1, 2, 3 and 4. When leaf lesions were used as the source of inoculum about 7 per cent of the infected plants produced variants. In table 3, where the source of inoculum was old stem lesions, about 30 per cent of the inoculated plants contained variants. Most of the variants were visible within 12 days of inoculation, often as primary lesions on the inoculated leaves. It is probable that they arose in the parent lesions but were unable to move away and consequently were not detected. The derivatives can be roughly classified by their symptoms on Turkish tobacco plants.

A. Those not becoming systemic.
   1) Local yellow type—N1, 13, 14, 18, 19, 43, 57, 83, 92, 95, 118, 120, 122

B. Those becoming systemic.
   1) Slow-moving yellow type—N50, 88, 103, 107, 123
   2) Fast-moving yellow type—N4, 5, 7, 8, 44, 49, 52, 56, 96, 97, 98, 116, 119, 121, 126
   3) Fast-moving severe yellow type—N41, 46, 100, 106, 111, 112, 114, 124

4) Non-distorting green type—N10, 16, 17, 93, 101
5) Distorting green type—N6, 15, 54, 55, 59, 60, 85, 89, 90, 94, 108, 115, 117, 125
6) Necrotic type—N2, 80.

The most noticeable characteristic of these derivatives is their wide divergence as to type. They cover a range similar to that exhibited by Jensen's (7) derivatives from tobacco-mosaic virus No. 1, exclusive of the green mosaics. His method of experimentation would necessarily exclude these. There is a high proportion of yellow mosaics that do not become systemic and are difficult to transmit. They differ only slightly from J–14, causing yellowing in tobacco, which is often followed in a few days by necrosis of the yellowed lesion. No special emphasis should, however, be attached to their close resemblance to strain J–14, since the green-mosaic viruses also frequently give rise to mutants of the localized yellow type.

The slow-moving, yellow strains N50, 103, and 107, are very similar to Jensen's (7) virus No. 111, which he described as a moderately slow-moving, yellow-spotting strain in tobacco. They do not cause vein-clearing symptoms nor a typical mottle. Secondary lesions show as isolated yellow spots with green centers or as a yellowing that spreads out from the veins. The strains are not highly infectious. Strain N88, which is distinctly different from the other members of the group, will be discussed later.

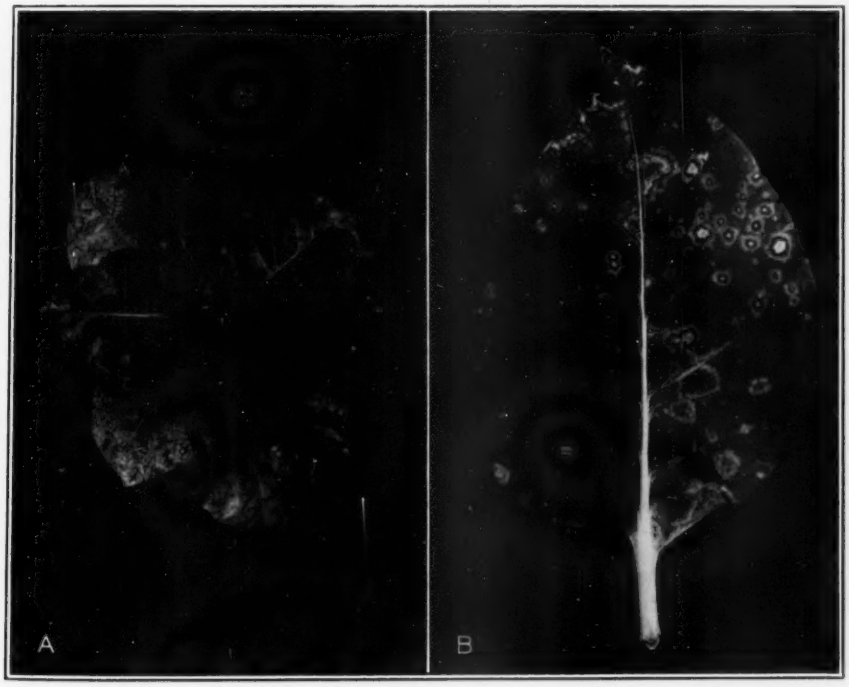

FIG. 1. Symptoms produced in tobacco by strain N4. A. Twenty days after inoculation. The plant is in the "recovery" stage, and subsequent invasion of the young leaves is beginning. B. One of these leaves, 15 days later, illustrating the formation of irregular yellow and green rings. (Photograph by J. A. Carlile.)

The fast-moving yellow types can be conveniently divided into classes on the basis of their severity on tobacco and other hosts. The less severe type shows a yellow mottle with some distortion of the leaves and a tendency to form yellow and green rings on tobacco (Fig. 1, B). There is little, if any, necrosis of the yellowed leaves. It has been possible to separate some of the viruses of this group on the basis of their symptoms in tomato and *Nicotiana sylvestris,* while others are so closely similar that it has not been possible to distinguish between them.

The severe, yellow-type strains cause first an intense yellowing of the leaf, which is followed rapidly by necrosis of the tissue (Fig. 2, C). If tobacco plants 4–6 inches high are used, the stems die usually near the base, and the young stunted leaves show a mixture of green, yellow and necrotic tissue. If larger plants are used, stem lesions are slower in appearing and the plants suffer less damage. On tomato the severe mosaics cause primary and secondary necrosis (Fig. 2, A, B), killing young plants within a short time. Jensen's (7) severe yellow mosaic variant, J–14D1, is similar to these strains in its action on tobacco and tomato.

Little attention has been paid to the green-mottling variants beyond dividing them into two groups on the basis of their ability or inability to cause distortion in tobacco.

Necrotic Type of Turkish Tobacco: Strain N2. Since no strain of tobacco-mosaic virus has been previously reported to cause systemic necrosis in Turkish tobacco, the symptoms produced by strain N2 will be briefly described. The symptoms were visible in 10 days in 4 plants of a December 3 set, inoculated from the same parent lesion. Subsequent tests on tobacco, tomato, *Nicotiana sylvestris,* and *N. glutinosa* showed that only one strain was involved. In Turkish tobacco, necrotic primary lesions appeared in 4–5 days (Fig. 3, A). Under some conditions the lesions were at first yellow, but they broke down rapidly. When vigorous plants up to about 6 inches in height were used, the virus almost always became systemic. When older plants were inoculated, the infection frequently remained localized. The onset of systemic infection generally appeared in the form of a few large yellow or necrotic spots, frequently connected with the midrib or with one of the larger veins of the young leaves. Necrosis of the yellow tissue occurred within a few days. In a later stage the most striking effect was necrosis of the veins, especially the midribs, of the young leaves, often with little or no destruction of the contiguous leaf tissue (Fig. 3, B). This resulted in a curious downbending and twisting of the top leaves and was characteristic of the disease. Stem lesions occurred in plants diseased for about 3 weeks and most commonly developed near the base of the stem. They were much lighter in color than those produced by strain J–14.

In *Nicotiana sylvestris,* strain N2 caused primary necrotic lesions in 5 days. The virus did not always become systemic, especially if mature leaves were inoculated. When systemic invasion occurred, the veins and particularly the midribs were killed.

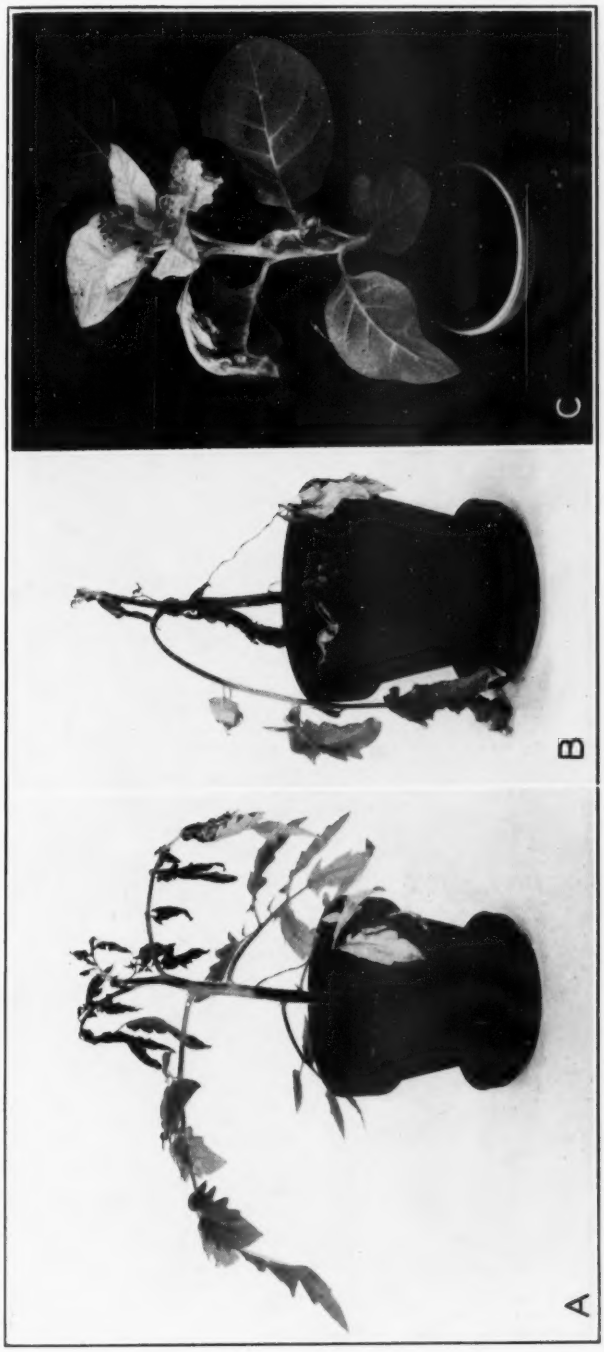

FIG. 2. Symptoms produced by the severe, yellow-type strains in tomato and tobacco. A. Strain K13 in tomato 12 days after inoculation. B. Strain N41 in tomato 14 days after inoculation. C. Strain N41 in tobacco 12 days after inoculation. (Photograph by J. A. Carlile.)

FIG. 3. The primary (A) and secondary (B) symptoms produced in tobacco by strain N2. Note in (B) necrosis of midribs of some of the upper leaves. (Photograph by J. A. Carlile.)

In tomato the first signs of infection were primary necrotic lesions, followed in a few days by secondary leaf spotting and petiole flecking. Within about 2 weeks the young leaves, the inoculated leaf and the large leaves on the same side as the inoculated one, became completely necrotic. Stem lesions did not invariably occur, but, when they did, the stem below the cotyledonary leaves was often killed. The growing-point seldom was destroyed, as usually happened after infection with Jensen's derivative, J–14D1. Otherwise, its action on tomato was similar.

Comparison of Mutants. In the process of maintaining strain J–14 over a period of about 18 months, the virus was transferred serially to a considerable number of tobacco plants by Dr. Kunkel. The plants were held in an actively growing condition for long periods of time. Some developed systemic infections of various types. The variants isolated from such infections were made available for comparison with those obtained in this work.

From their symptoms on tobacco this second group of mutants can conveniently be divided into a number of classes:

A. Those not becoming systemic
   1) Yellow type—none
B. Those becoming systemic
   1) Slow-moving yellow type—K16
   2) Fast-moving yellow type—K1, 5, 9, 11, 12, and 14
   3) Fast-moving severe yellow type—K17, 13
   4) Nondistorting green type—K10
   5) Distorting green type—K2, 3, 4, 6, 7, 8, and 18
   6) Greenish-yellow type forming necrotic rings—K15

The first obvious difference between the two sets is the absence of the local, yellow type in the second set. In maintaining J–14, nonsystemic derivatives were not looked for and, thus, only systemic variants were collected. In the first set the local, yellow derivatives appeared about 6 days after inoculation and the presumption was that they were already present in the inoculum. If the necrotic lesions of J–14 occur near the base of the leaf, it seems unlikely that these local yellow mutants can enter the stem and reach the leaf above. It is possible that some of the mutations recorded were not derived directly from J–14 but from a slow-moving mutant of J–14.

The systemic strains, apart from K15, fall naturally into the same types as the strains subsequently isolated by the writer. The slow-moving yellow strain, K16, looks very like the strains N50, N103, and N107 in tobacco. On mature leaves of tomato they all produce concentric rings of yellow and green tissue. Figure 4 illustrates the symptoms produced by strain K16 on the inoculated leaf of a tomato plant.

Among the fast-moving yellow types many strains of the two sets have been indistinguishable from each other by their symptoms on tobacco, tomato, *Nicotiana sylvestris* and *N. glutinosa* plants growing at the ordinary

FIG. 4. Concentric rings of yellow and green tissue produced on the inoculated leaf of tomato by K16, one of the slow-moving, yellow-type strains. (Photograph by J. A. Carlile.)

greenhouse temperature (75° F.). During the summer, when the greenhouse temperature (13) in the daytime was frequently above 90° F., the following strains could not be differentiated: K1, K5, K9, K11, K12, K14, N8, and N4. Of the severe fast-moving strains, K13 looks identical on tobacco and tomato plants with several of the severe strains that were subsequently isolated.

The strain that forms necrotic rings in tobacco finds no parallel in the first set and will be described briefly. The disease it produces resembles closely that described by Johnson (8) as "ring mosaic."

Necrotic-ring Type: Strain K15. On Turkish tobacco greenish-yellow, primary lesions appeared 4 days after inoculation. Each lesion then de-

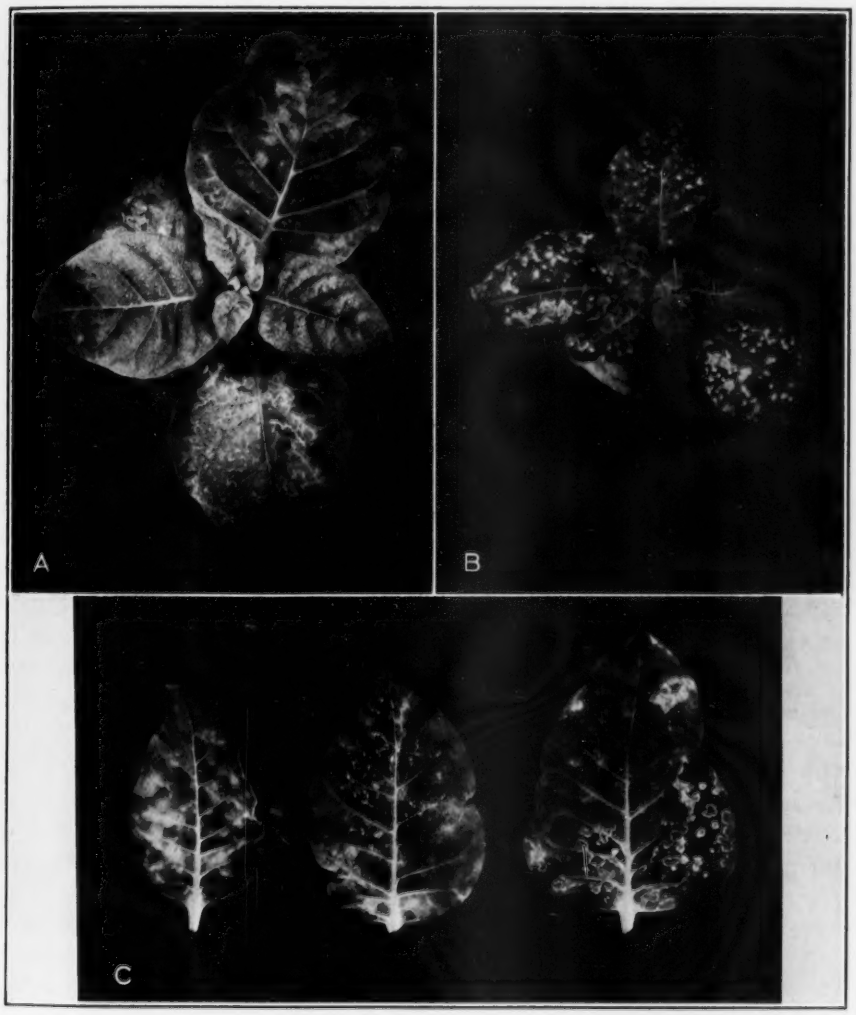

FIG. 5. Symptoms produced by strain K15 in tobacco. A. Primary lesions with necrotic rings on the lower leaves. B. Secondary necrotic spotting. C. Stages in the formation of necrotic rings. (Photograph by J. A. Carlile.)

veloped a ring of dead tissue at its periphery (Fig. 5, A). In some cases a necrotic center as well as margin was evident. Eight days after inoculation the whole lesion became necrotic. In other cases necrotic lesions were the first signs of infection.

Systemic infection appeared first as a clearing-of-veins followed later by a greenish-yellow mottle on the young leaves. On the leaves already fully developed, infection might follow the veins to give oak-leaf patterns of yellowed tissue edged with necrotic, or, when infection occurred in isolated spots, each large spot might at first be surrounded by a ring of necrotic tissue before the lesion dried out (Fig. 5, B).

Necrosis of the young infected tissues started some days after the mottle when the leaves began to expand. In general, the necrosis began at the edge of the yellowed tissue and spread inwards. Sometimes it formed irregular rings; at other times it was a wavy line only a few millimeters long (Fig. 5, C). As the plant grew, the lower leaves shriveled up and died, while the size of the upper leaves was greatly reduced. Stem lesions usually did not occur.

Nicotiana sylvestris was not highly susceptible to infection with K15. In 5 days large greenish-yellow lesions with necrotic edges appeared. These lesions rapidly became completely necrotic. Systemic symptoms appeared as a necrotic spotting connected with the veins of the young leaves. Initially the necrosis might assume a ring form. The plants were killed in about 30 days.

On tomato the symptom picture was much like that described for N2.

Infectivity. It was noticed that the different yellow strains varied greatly in their infectivity. There seemed to be a correlation between the rapidity of movement in tobacco and tomato on the one hand and infectivity on the other. For example, the local, yellow-type strains have a low infectivity; the slow-moving yellow strains seemed to be intermediate in infectivity between the local, yellow strains and the fast-moving, yellow strains. An experiment was run to check this observation, using a typical strain from each class. As the strains formed distinct, yellow lesions in tobacco, it was used as the test plant. Each dilution of virus was inoculated by rubbing with a sterile pad to 5 leaves of each of 2 tobacco plants. The lesions were counted on the fifth day. The results are recorded in the graph (Fig. 6). Both strains that become systemic were very much more infectious than the local, yellow strain. The fast-moving, yellow strain was about 10 times as infectious as the slow-moving, yellow strain. No adequate explanation for the correlation between infectivity and rate of movement has been found.

Frequency of Origin. Table 5 shows the frequency with which strains of the different types have been isolated. It can be said that, in comparison with the other strains, the slow-moving, yellow-type and the nondistorting green strains arise infrequently. Since the local, yellow-type strains are much less infectious than any of the other types, it is presumed that they

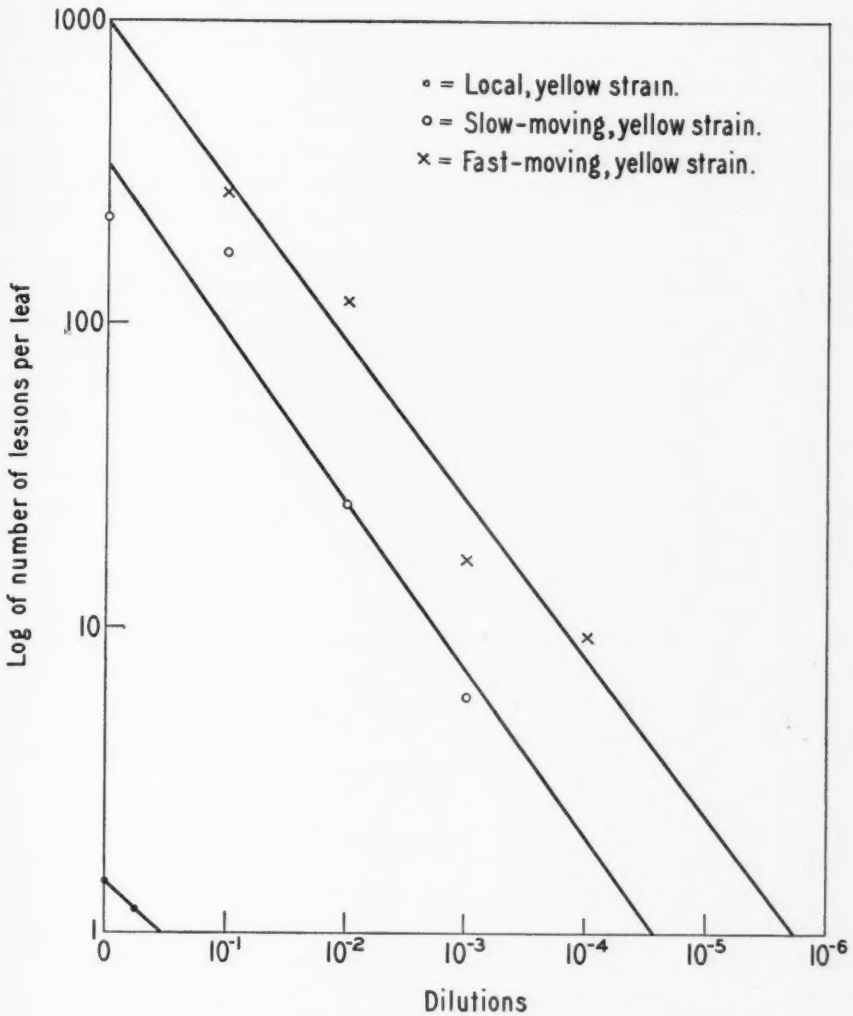

FIG. 6. Comparison of infectivity of three yellow strains that differ in the rates at which they move in the tobacco plant.

were more difficult to isolate. In view of this the large number obtained probably means that they were the most frequent type arising. The high

TABLE 5.—*Frequency of isolation of strains of the various classes*

Strain type	Number of isolates		Total number of isolates
	N	K	
Local, yellow	13	....	13
Slow-moving, yellow	5	1	6
Fast-moving, yellow	15	6	21
Severe, systemic	10	3	13
Non-distorting, green	5	1	6
Distorting, green	14	7	21

proportion of severe, systemic strains that cause necrosis of leaf and stem in tomato is interesting because of the severity of the parent strain and the fact that Jensen obtained none of them among the strains isolated from the ordinary tobacco-mosaic virus. The fast-moving yellow and the distorting, green strains are comparable in infectivity and were obtained in about equal numbers. Consequently, it is thought that they arose with about the same frequency.

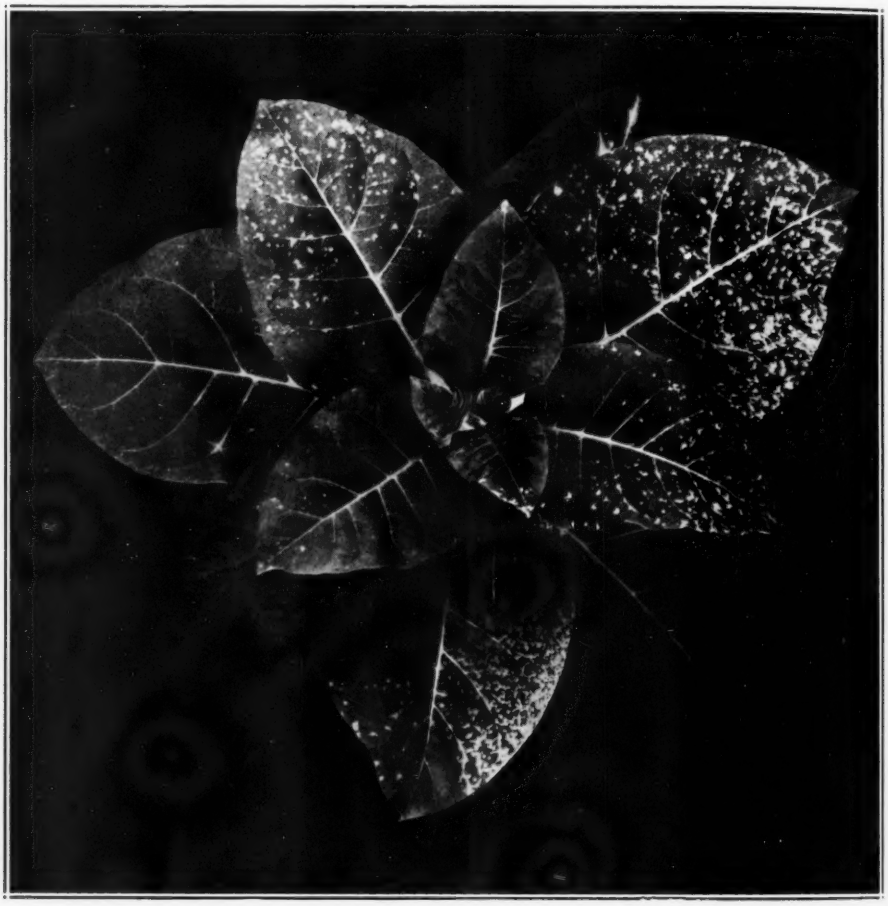

FIG. 7. Symptoms produced in tobacco by strain N88. (Photograph by J. A. Carlile.)

From the point of view of the frequency of origin, strain N88 is exceptionally interesting. In common with strain J104 it produced a mild, yellow spotting in tobacco (Fig. 7), was not highly infectious, and did not move readily. In *Nicotiana glutinosa* both strains produced minute, necrotic lesions that later developed a yellow halo. Jensen (7, p. 78) figures the lesions caused by strain J104 on *N. glutinosa* leaves. From the large number of variants isolated from the common tobacco-mosaic virus and its

derivatives, only two have been reported to form the atypical minute, local lesions in *N. glutinosa*.

DISCUSSION

In this study it has been observed that most of the variants arose a short time (*ca.* 10 days) after inoculation and could first be seen as primary lesions upon the rubbed leaves. That variants arose early, and frequently were visible on the inoculated leaf, means either that they were already present in the inoculum or that they were introduced as contaminants during transmisison. The evidence, accumulated during this investigation, from check plants and from a thorough testing of the methods of transmission, renders it highly improbable that the variants represent contaminations. Further evidence that they are mutants is furnished by the character of the strains themselves. For example, the necrotic and some of the yellow-type strains are seldom found in nature and are thus unlikely to be contaminants, though some of the highly infectious green or yellow mosaics might be. Also, it has been established that the strains isolated are all related to the tobacco-mosaic virus group. On *Nicotiana glutinosa* none, except strain N88, failed to respond with the production of characteristic necrotic lesions. The following strains: N2, 4, 7, 9, 12, 50, 88 and K9, 12, 13, 14, and 15 all reacted positively when tested serologically against tobacco-mosaic virus serum. The author is indebted to Dr. L. M. Black for carrying out the tests. Strain J–14 and the local yellow-type strains could not be recovered from dried leaf material, but they produced the characteristic local lesions in *N. glutinosa*, and no infection was obtained when they were inoculated to plants already infected with the ordinary tobacco mosaic, so that they are undoubtedly strains of the tobacco-mosaic virus (2, 10).

As a class, the mutants represent a wide range of strains varying in their infectivity, their ability to move, their action on the chlorophyll mechanism and the destruction of tissues. A few significant results emerge from this study. The ability to cause tissue destruction in tobacco and tomato is not necessarily connected with low infectivity and inability to become systemic. For example, N2 and N80 cause primary and secondary necrosis in tobacco. Both can be transmitted readily without the aid of carborundum, and infectivity is still retained by N2 at a dilution of 1 : 1000. In tomato N2, 80, 41, 46, 100, 106, 111, 112, 114, 124, and K13 and 15 all cause secondary necrosis and are highly infectious. On the other hand, infectivity, in the cases observed, is certainly correlated with the ability to move out of the inoculated (7) leaf when tested on tobacco or tomato. The yellow type that does not become systemic, and J–14 itself, have an extremely low infectivity, while the slow-moving, yellow type is intermediate in infectivity between the local and the fast-moving, yellow types. No exceptions to this rule have been reported in the tobacco-mosaic virus group.

Another point of interest is the comparatively large number of mutants that are extremely severe on tobacco and tomato, and, in this respect, resemble J–14 but differ from the mutants derived by Jensen (5, 6, 7) from the common tobacco-mosaic virus. Holmes (3) had previously shown a similar relationship between strains of the tobacco-mosaic virus group and their derivatives. He collected yellow mutants from a "masked" and a "distorting" strain. The "masked" strain was conspicuously less able than the "distorting" to invade the young tissues of Turkish tobacco. In all the derivatives of the "masked" strain this character was reproduced, but it was absent from most of the derivatives of the "distorting" strain. Despite the definite tendency of the derivatives to resemble the parents closely in this character, the variants as a whole differed widely from each other and from the parent strain. This is equally true of the derivatives from J–14.

From the reaction of certain classes of derivatives from J–14 in Turkish tobacco, tomato and *Nicotiana sylvestris* the following table has been constructed:

Strain type	Turkish tobacco	Tomato	*N. sylvestris*
A (N2)	Necrosis	Necrosis	Necrosis
B (N41)	Yellow	Necrosis	Necrosis
C (N5, 49)	Yellow	Yellow	Necrosis
D (N4, 7)	Yellow	Yellow	Yellow

It is apparent that there is a progressive increase of tissue sensitivity to strains of tobacco-mosaic virus from Turkish tobacco through tomato to *N. sylvestris*. Strains (type A) that caused necrosis in Turkish tobacco never failed to kill tomato and *N. sylvestris* tissue. Strains (type B) that destroyed tomato tissue always caused necrosis in *N. sylvestris*, while strains (type C, represented by N5, 9 and 49) that were comparatively mild in tobacco and tomato still destroyed *N. sylvestris* tissue. There is a fourth type, D, in which the strains do not cause necrosis in any of the hosts. Schematically, this progressive diminution in severity of the different virus strains may be represented as follows:

$$A = B + = C \ddagger = D \ddagger \text{ where } + = \text{an "intensifying" factor.}$$

Since the host plants are not known to differ from each other in sensitivity to the virus strains by single genetic factors, the "intensifying" factor postulated is not considered to represent a single genetic factor.

Some evidence has accumulated that suggests the possible repeated occurrence of the same mutation. Among the fast-moving, yellow types, certain of the strains obtained at different times in both the K and N series cannot be distinguished from each other by their symptoms on tobacco, tomato, *Nicotiana sylvestris* and *N. glutinosa*. From a consideration of the technique and controls, it seems very unlikely that they represent recurrent contaminations. Within the other groups obtained there are many strains

that resemble each other closely. Little attempt has been made to distinguish between them. However, apart from these mutants, the evidence gathered from the systemic yellow-mosaic types tends to show, at least within the limits to which identity can be established, that the same mutation does occur repeatedly.

It is interesting to note that one of the more severe local, yellow strains, N92, which had previously been passed twice through tobacco by single-lesion transfer, when inoculated to 10 tobacco plants produced about 200 yellow and 2 necrotic lesions. Upon subsequent transfer of the necrotic lesions to tobacco only local, necrotic lesions resulted. These lesions were identical with those produced by J–14 in tobacco and the strain could not be distinguished from J–14 in other ways. If it is the same strain, this is a case of a reversion.

Bawden and Pirie (1) and Loring (11) have demonstrated that the tobacco-mosaic virus protein is a nucleoprotein. It is known that the hereditary material in plants and animals is concentrated in the nucleus, which contains nucleoproteins. The different heritable factors are carried on the chromosomes at specific loci. A factor may change spontaneously to give a new factor. In this characteristic the virus protein resembles the gene or unit of reproduction in the hereditary mechanism. It is possible that the study of the heritable variations of the virus protein will aid in the final understanding of the structure of the gene.

SUMMARY

Variants from an unusual strain of tobacco-mosaic virus, Jensen's J–14, have been collected and examined. J–14 has a low power of infectivity, produces in Turkish tobacco local necrotic lesions, and never becomes systemic. The derivatives are widely scattered throughout the possible symptom types in tobacco and include local yellow types, slow-moving yellow types, fast-moving yellow and green types of varying severity, systemic necrotic types, and a strain that forms necrotic rings.

Twelve distinct strains cause primary and secondary necrosis in tomato, and thus resemble J–14 in their ability to destroy tomato tissues. Strains of this type occur rarely in derivatives of the green mosaics.

In tobacco and tomato the ability to destroy tissues seems to be independent of the ability to become systemic, but infectivity is correlated with the ability to move.

Many of the mutants, obtained at different times, cannot be distinguished from each other by their symptoms on Turkish tobacco, tomato, *Nicotiana sylvestris* and *N. glutinosa*.

The strains that cause necrosis in Turkish tobacco are regarded as having the full complement of factors for necrosis. Strains that destroy the tissues of more sensitive plants but not those of tobacco are thought to lack some of these factors. By using a range of host plants differing in their

sensitivity, it has been possible to recognize within the different strains of virus several factors for necrosis.

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TWIG CANKERS OF ASIATIC CHESTNUTS IN THE EASTERN UNITED STATES

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INTRODUCTION

The Chinese chestnut (*Castanea mollissima* Bl.) was known in the United States to a slight extent before the Civil War and the Japanese chestnut (*C. crenata* Sieb. and Zucc.; syn. *C. japonica* Bl.) was introduced about a decade after its close (14). Subsequently a number of Japanese chestnuts were grown rather successfully in orchards. The Chinese chestnut was introduced into the United States by the Department of Agriculture in 1907 (3). There was a great stimulus to their introduction when it became generally evident that the commercial stands of our valuable American species [*C. dentata* (Marsh.) Bork.] were doomed by chestnut blight. In the past 10 years Asiatic species, which are resistant to chestnut blight, have been introduced for extensive trial in forest and orchard plantings. Bedwell (1) described many of the factors affecting the survival of these Asiatic chestnuts in forest plantations, the most important of which was dieback induced by drought, twig blights, low temperatures, or planting injury. He found various fungi in association with the twig blights and described one of them caused by *Phomopsis* sp. (2).

The writer also has isolated a number of fungi from dying Asiatic chestnuts, but he has confined most of his observations and studies to those found associated with definite cankers. Three of these (*Cryptodiaporthe castanea* (Tul.) Wehmeyer, *Botryosphaeria ribis chromogena* G. and D., and *Diplodia* sp.) have been proved to cause cankers. The discussion presented herein is based on field observations and experiments during the past 6 years and is centered around these three fungi.

SYMPTOMS OF TWIG CANKERS

Because of the similarity in the external symptoms of the three diseases and the inability to distinguish them definitely by macroscopic examination, the description of symptoms applies to all three. However, the description is specifically based on the disease caused by *Cryptodiaporthe castanea*. This is the one most commonly found and has already been reported by the writer[1] as occurring on Asiatic chestnuts in this country.

The cankers or diebacks occur on trunk, limbs, or twigs. In the vicinity of Washington, D. C., the youngest incipient infections are observed in January and February and are manifested as small areas of diseased bark, a shade darker than healthy bark. The causal fungus develops rapidly, kills the bark and the cambium, invades the sapwood, and induces a brown dis-

[1] Fowler, Marvin E. *Cryptodiaporthe castanea* on Asiatic chestnuts. Plant Disease Reporter 22: 69. 1938.

coloration of the host tissue. In early spring when the fungus is actively growing, the discoloration of the sapwood usually extends beyond that of the overlying bark, but later the entire area of the bark overlying this infected tissue discolors and dies.

During a single season the fungus may girdle a small twig, kill it to its junction with a larger branch, and invade that, too. The leaves on girdled branches usually wilt without yellowing and gradually brown and die. The killing occurs most rapidly from February to June, but some killing may occur throughout the year, especially on trees weakened by other factors. If a stem has not been completely encircled by early summer, the formation of callous tissue begins, sometimes causing the bark to split along the edges of the diseased area and a more pronounced canker results (Fig. 1, A). The long axis of the cankers parallels that of the stem. The cankers are sometimes completely healed over during the summer, but, frequently, killing is resumed the following year, causing the cankers to enlarge and often to girdle the stem (Fig. 1, B).

In a nursery at Glenn Dale, Maryland, 125 incipient cankers on Japanese and Chinese chestnuts were selected for the purpose of determining the avenue of entrance of *Cryptodiaporthe castanea*. Of these cankers 85 per cent had small dead twigs in their centers, indicating that the infection had occurred through those twigs or at their bases, 6 per cent had a dead bud in the center of the canker, 8 per cent had developed around pruning wounds, and 1 per cent had a live bud near the center of the canker.

All 3 fungi fruit readily in the dead tissue, and a microscopic examination of these fructifications, or of cultures made from them or from diseased tissue, is necessary in order to distinguish them.

INOCULATIONS

Inoculations were made on healthy, vigorous, potted 2-year-old chestnuts in an effort to determine whether the fungi found associated with the cankers and dieback twigs were parasitic. All the inoculations were made in the spring when the plants were either dormant, with swollen buds, or with small leaves. A number of fungi were tested, but the results are presented only for the three found to be somewhat parasitic and for the comparable check plants. The failure of the other fungi to parasitize may possibly be due to the conditions of the experiment and may not necessarily mean that they are entirely saprophytic under all conditions.

These inoculation experiments were conducted each spring in the pathological greenhouses at Washington, D. C., from 1932 to 1936. *Castanea crenata* and *C. mollissima* were inoculated each year; *C. pumila* Mill., in 1932; and *C. henryi* Rehd. and Wils., in 1936. Several inoculations were made on both the main stem and the lateral branches of each tree. All check trees were treated just like the inoculated ones, except that sterile agar was used in the wounds on some and nothing in those on others.

The stems to be inoculated were first cleaned by swabbing with 95 per

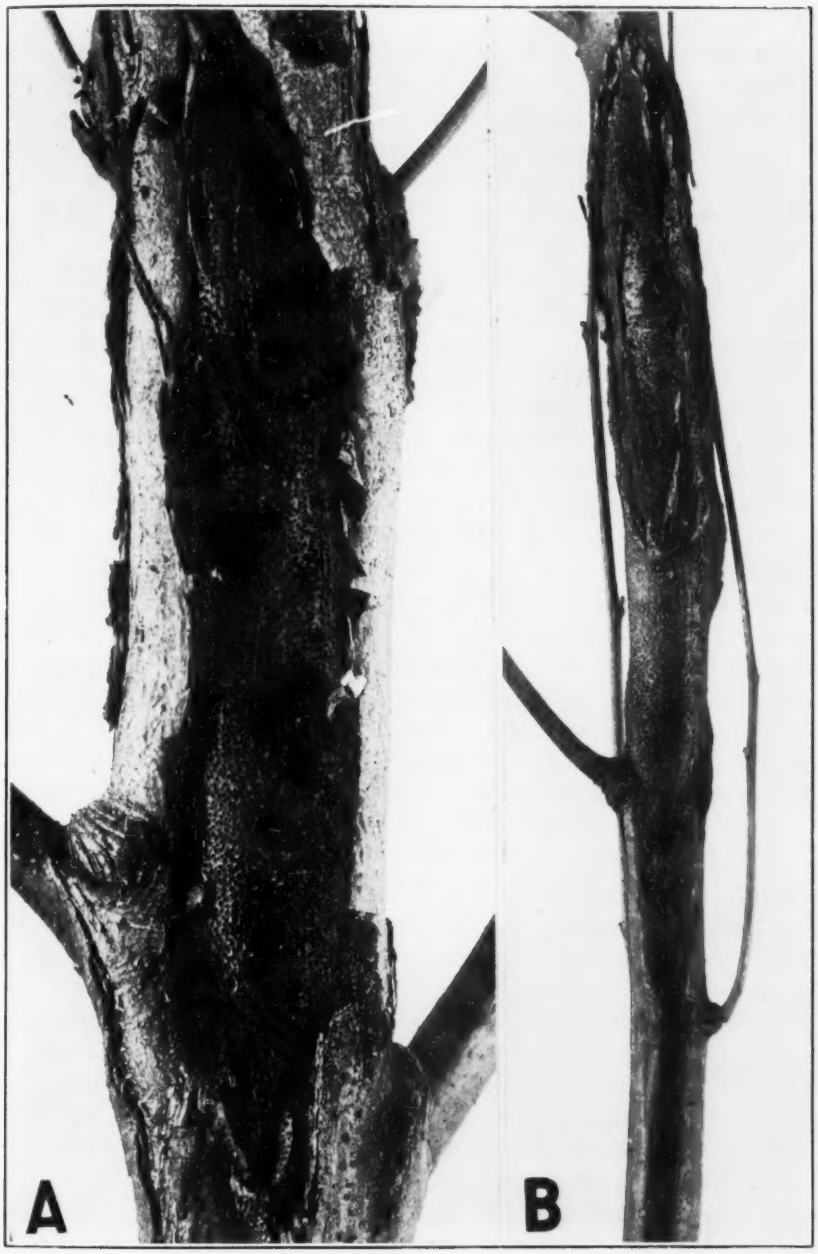

FIG. 1. Typical cankers caused by *Cryptodiaporthe castanea* on Japanese chestnut. A. Old canker with abundant perithecia on dead bark. B. Canker from which the fungus had spread beyond the original callous tissue in the second year and had girdled the stem. Perithecia occur in center of canker and pycnidia on recently killed tissue below. Remains of a small twig can be seen in the center of each canker. Approximately × 1.

cent alcohol, which was immediately burned off, or with 1:1000 mercuric chloride. A small incision was then made through the bark and cambium into the sapwood with a sterile scalpel. Into these wounds was inserted the surface growth of the fungi produced in culture. These were made from diseased tissue and from single conidia of all 3 fungi and from single asci of the *Cryptodiaporthe* and the *Botryosphaeria*. The wounded places on all trees were wrapped with moistened sterile cotton and the plants were placed in an "iceless refrigerator," like that described by Hunt (11), for 24 to 48 hours before being transferred to greenhouse benches. The cotton over the wounds was kept moist for one to two weeks before being removed.

As results of the inoculations did not differ significantly in different years, only the totals are shown in table 1. In general, plants inoculated while dormant were more easily infected by all three fungi than those with swollen buds or small leaves, but they were not always most severely damaged. *Castanea crenata* was more easily infected and more severely damaged by each of the 3 fungi than was *C. mollissima*. The number of inoculations on *C. henryi* and *C. pumila* was not sufficient to permit drawing definite conclusions on the degree of parasitism on them.

All inoculations were examined every few days in order to observe the killing of the tissue immediately around the inoculation wounds before the fungus had spread sufficiently to girdle the twigs. Some twigs were girdled within 2 weeks after being inoculated, while others, especially those of larger diameter, were not girdled for several weeks. After being girdled by the fungus, many seedlings sprouted at the base. On some twigs the fungi killed the tissue adjacent to the wounds, forming incipient cankers that continued to enlarge for several weeks. Callous tissue later formed around many of these affected areas and more pronounced cankers resulted. In many cases the callus covered the wounds. Pycnidia were formed on many lesions induced by inoculations made with cultures from diseased tissue and single conidia of the *Cryptodiaporthe, Botryosphaeria,* and *Diplodia* and from single-ascus cultures of the first two. Microscopic examinations of these pycnidia showed them to be the fructifications of the imperfect stage of the fungi used. Single-conidium cultures were obtained and compared with the cultures from the original specimens. Both the original cultures and those recovered from inoculations were used in subsequent inoculations and again produced the disease. Each of the 3 fungi were recovered from these inoculations and again successfully inoculated into and recovered from *Castanea mollissima* and *C. crenata* trees.

The checks consisted of 162 wounds on 37 *Castanea mollissima* trees, 213 on 50 *C. crenata,* 16 on 4 *C. pumila,* and 39 on 8 *C. henryi.* Some of these trees were dormant, others had swollen buds, and others small leaves. In no case did a canker form around the wound on a check tree. In addition, a number of trees were inoculated with other fungi cultured from dieback twigs. These inoculations might be considered as further checks, as no cankers resulted from them.

TABLE 1.—*Inoculation of Castanea spp. with Cryptodiaporthe, Botryosphaeria, and Diplodia, from 1932 to 1936*

Inoculating fungus, name, and condition of host	Trees inoculated	Trees infected	Inoculations		
			Number	Percentage infected	Percentage of infections girdling
	Number	*Per cent*			
Cryptodiaporthe castanea					
Castanea crenata—					
Dormant	15	100	57	91	63
With swollen buds	26	88	107	50	51
With small leaves	9	67	34	47	6
Total	50	88	198	61	50
Castanea mollissima—					
Dormant	13	100	52	67	37
With swollen buds	23	52	98	37	39
With small leaves	13	69	51	55	43
Total	49	69	201	49	39
Castanea henryi	4	100	17	100	88
Castanea pumila	6	83	22	64	57
Botryosphaeria ribis chromogena					
Castanea crenata—					
Dormant	5	100	19	100	47
With swollen buds	16	94	73	88	61
With small leaves	2	100	10	60	17
Total	23	96	102	87	55
Castanea mollissima—					
Dormant	3	100	12	100	33
With swollen buds	15	100	72	83	38
With small leaves	6	100	30	80	42
Total	24	100	114	84	39
Castanea henryi	4	100	20	95	68
Castanea pumila	1	100	4	100	50
Diplodia sp.					
Castanea crenata—					
Dormant	6	100	26	85	50
With swollen buds	17	94	85	71	42
With small leaves	7	86	31	55	24
Total	30	93	142	70	40
Castanea mollissima—					
Dormant	4	100	13	69	56
With swollen buds	14	71	63	56	29
With small leaves	12	83	52	67	34
Total	30	80	128	62	34
Castanea henryi	4	50	19	42	50
Castanea pumila	2	100	8	88	29

ISOLATION AND IDENTIFICATION OF CAUSAL ORGANISMS

At first it was the writer's practice to make several tissue isolations from each diseased specimen and to make single-spore isolations of the fungi fruiting on these specimens for comparison. Hundreds of cultures have been made from 1931 to date, and it was found that the causal organisms

were easily isolated from tissue taken under aseptic conditions from the extreme edges of actively growing cankers or from single or mass plantings of spores. The attempts to isolate them from dieback twigs were often hampered by the presence of other fungi. Isolations were made on poured plates of corn-meal, malt, prune, and potato-dextrose agar with equal success.

Cryptodiaporthe castanea (Tul.) Wehmeyer

The fungus found most frequently forms numerous small black raised pustules within the diseased bark from April to May. About 3 weeks after the pustules form, the overlying bark breaks and the pustules become erumpent. Microscopic examinations reveal that this breaking of the bark occurs at approximately the same time as conidia are produced. The conidia are typically 2-cell, slightly unequal, fusoid, straight or slightly curved, hyaline, 10–13 × 2.5–3.5 μ. Some 1-cell conidia are usually present. This form of the fungus was identified as *Cytodiplospora castaneae* Oud. Perithecia later form in groups near the basal stroma of the imperfect stage in the summer and by midsummer have pushed their beak-like ostioles up through the ectostroma until they are barely erumpent through the bark. The asci are 35–50 × 6–9 μ and the ascospores 10–15 × 2–3 μ. The ascospores are very similar to the conidia, except that they are slightly larger and may be definitely distinguished by the very short hyaline cylindric appendage usually found at each end. This form of the fungus was identified as *Cryptodiaporthe castanea* (Tul.) Wehmeyer [*Diaporthe castanea* (Tul.) Sacc.] and agrees with the description given by Wehmeyer (18). It is distinguished from *Diaporthe* by the absence of blackened zones in the substratum. Cultures made from single conidia, asci, and ascospores were identical in appearance, and, after growing in test tubes for several weeks, produced identical conidia.

Prillieux and Delacroix (13) described and illustrated *Diplodina castaneae* on European chestnut, which has been considered by later workers to be the same as *Cytodiplospora castaneae* described by Oudemans (12). They, however, described 1-septate, 6–7 × 1–1.5 μ conidia borne in separate and distinct pycnidia, while Oudemans described 1-septate, 9–12 × 2½–3 μ conidia produced in compartments arranged in a circle about a parenchymatous axis. The fructifications of the fungus studied by the writer vary, but, in general, agree with Oudemans's description of *Cytodiplospora*. Dufrénoy (8) illustrated *Cytodiplospora castaneae* with 2-cell conidia. Wehmeyer (18) did not describe the pycnidial stage of *Cryptodiaporthe castanea* in detail and made no mention of 2-cell conidia. He listed *Cytispora castanea* Sacc., *Fusicoccum castaneum* Sacc., and *Malacostroma castaneum* (Sacc.) Hohn under its conidial connections. Although Défago (5) stated that he proved the conidial connection of *Cryptodiaporthe castanea* based on single-ascospore and single-conidium cultures, his description and illustrations are of 1-cell conidia only. Grove (10) described the conidia of *Fusicoccum castaneum* Sacc. (*Cytodiplospora castaneae* Oud.) as being

1-septate when fully mature and stated that the likeness of these conidia to the ascospores of the perfect stage was so great that it was not until he had seen the former in large numbers seated upon their sporophores that he was satisfied that they were not escaped ascospores devoid of their row of guttules. This observation by Grove on the morphology is confirmed by the writer. In addition, the writer established the conidial connection by comparing single-ascospore, single-conidium and single-ascus cultures. The short, cylindric, hyaline appendage often observed at each end of mature ascospores has not been observed on conidia, although numerous examinations have been made.

Botryosphaeria ribis chromogena G. and D.

On August 28, 1931, a fungus that appeared to be *Botryosphaeria ribis chromogena* G. and D. was collected on a Japanese chestnut at Arlington Farm, Virginia, and in April, 1934, one that appeared to be *Dothiorella ribis* was collected on Chinese chestnuts at Natchez, Miss. *D. ribis* had previously been collected on dead American chestnut twigs at several localities. Single-ascospore and single-conidium cultures made from the collections on Asiatic chestnuts were identical and both produced conidia. When grown on corn meal in 100-cc. Erlenmeyer flasks, numerous knob-like stromatic bodies were produced and the medium was colored purplish pink. This fungus is a known parasite of currants, producing the disease called currant cane blight, and it has been distinguished from a similar form by its ability to color starch paste purplish pink. The ascospores on chestnut are $15–22 \times 5–7.5\ \mu$ and the conidia, $14–28 \times 5–8\ \mu$ and are both within the range given for this species. Although Shear, Stevens, and Wilcox (15) stated that single perithecia are often found and that the size of the stromata varies with the host and thickness of the bark, both the perithecia and pycnidia found on chestnut occur in groups in a stroma.

As a further means of determining whether the chestnut organism is identical with the currant cane blight fungus, Dr. Neil E. Stevens suggested that inoculations be made on currants susceptible to the currant cane blight. With this fungus 47 inoculations were made on 10 Fay's Prolific and 23 inoculations on 6 Cherry currants. The checks consisted of 36 wounds on 8 currants of the above-named varieties in which sterile agar was inserted. Further checks consisted of 32 wounds on 7 Cherry currants that were inoculated with *Cryptodiaporthe castanea* and 9 wounds on 2 Cherry currants inoculated with the chestnut *Diplodia*. Of the inoculations made with the chestnut *Botryosphaeria* 23 produced lesions on the Fay's Prolific and 14 on the Cherry currants. The *Dothiorella* stage of the fungus appeared on the killed areas. Single-conidium reisolations were made from these fructifications. No lesions were produced by the *Cryptodiaporthe* or *Diplodia* inoculations or on the check plants. This gives additional evidence that the chestnut *Botryosphaeria* is identical with the one on currants. Smith (16) successfully inoculated *Castanea* sp. with *Botryosphaeria ribis chromogena* isolated from citrus and walnut.

Diplodia sp.

In October, 1931, a specimen of Chinese chestnut, on which a species of *Diplodia* was fruiting, was collected at Jackson, Tenn. The black, globoid, erumpent, pycnidia of this fungus are sometimes produced singly, but usually in groups in a stroma in the outer layers of the bark. The conidia are sub-hyaline and 1-cell when first formed, but, upon maturity, many become 2-cell and dark. They are ovoid to elliptic and measure 18–26 × 11.5–16 μ. Single-conidium cultures produced conidia that agreed in size, color, and shape with those on the host. This fungus has not been specifically identified. On other collections of Asiatic chestnuts a *Diplodia* with conidia of a different size was fruiting. Inoculations made with cultures obtained from this gave negative results.

DISTRIBUTION AND DAMAGE

In the eastern United States, numerous collections have been made of cankers on Asiatic chestnuts, and *Cryptodiaporthe castanea* has been found to be the most commonly associated fungus. In some plantations no other fungus has been found fruiting on the dead tissue in the cankers. This fungus has been collected and identified on *Castanea* spp. from the following localities: Maine—Cape Elizabeth; New Hampshire—Raymond; Massachusetts—Petersham; Connecticut—Stamford; Rhode Island—Providence; New York—Castile; New Jersey—Newfoundland, Phillipsburg, Washington Crossing; Delaware—Mount Cuba; Pennsylvania—Bally, Barto, Erie, Lancaster, Tryonville; Maryland—Annapolis, Beltsville, Bigpool, Fort George G. Meade, Glenn Dale, Port Republic; District of Columbia; Virginia—Abingdon, Arlington, Buena Vista, Drewrys Bluff, Fairfax Station, Gordonsville, Natural Bridge, Warrenton; North Carolina—Andrews, Asheville, Busick, Chapel Hill, Durham, Franklin, Lake Logan, Morganton, Raleigh, Stillhouse Branch, Willets; Georgia—Albany, Jasper, Savannah; Alabama—Moulton, Muscle Shoals, Prattville; Tennessee—Byrdstown, Harriman, Jackson, Waverly; Ohio—Brecksville, Springfield, Wooster; Michigan—Battle Creek; Texas—Kirbyville. These collections include *Castanea mollissima*, *C. crenata*, and *C. henryi* in numerous localities and *C. sativa* Mill. from the District of Columbia, only.

This wide distribution of the fungus cannot be considered as evidence that the organism is native, as most of the plantations and orchards were established from trees grown in a few recently established nurseries. As the fungus has been found in some of these nurseries, it could have been distributed from them because, during the transplanting season, in early spring, many infections are incipient and could undoubtedly escape the attention of inspectors. However, the fungus was present in 3 widely separated Japanese chestnut orchards planted 30 to 40 years ago at Drewrys Bluff, Fairfax Station, and Gordonsville, Virginia, long before the establishment of the above-mentioned nurseries. Several years ago when the fungus was first observed in these plantations the appearance of the diseased

trees was such as to indicate that it had been present for some time. Furthermore, at that time additional plantings of Asiatic or European chestnuts had not been made within several miles of these orchards. The same fungus is also abundant on two 43-year-old Japanese chestnuts at Barto, Pa., in the vicinity of which no other Asiatic or European chestnuts have more recently been planted. The source of these infections has not been determined. Numerous examinations of twig fungi on native *Castanea* and *Quercus* spp. have not revealed the presence of any that resemble *Cryptodiaporthe castanea*.

This fungus has been known to occur on *Castanea sativa* in Europe for many years. Prillieux and Delacroix (13) observed a canker disease, which they called "Le Javart," on European chestnut in 1893 and named the associated fungus *Diplodina castaneae*. Oudemans (12) described what is apparently the same fungus as *Cytodiplospora castaneae* on stems of European chestnut collected in Holland in 1893. Later, Ducomet (7) studied the "Le Javart" disease and identified the fungus as *Cytodiplospora castaneae* Oud. This disease has been mentioned as occurring in Europe by other workers, including Foëx (9), Day (4), and Dufrénoy (8). Dufrénoy stated that it parasitized the bark of Japanese chestnut, weakened by transplanting or by some unfavorable factor. Grove (10) reported this fungus on dead twigs and branches of European chestnut in England and gave its distribution as France, Holland, Germany, and Italy. More recently, Défago (5) described in detail a disease of young European chestnuts in a nursery and attributed it to *Cryptodiaporthe castanea* (Tul.) Wehmeyer. He proved its parasitism by inoculations and considered the fungus to be capable of developing a canker that is often completely stopped by the formation of callous tissue unless external conditions lower the resistance of the plant.

In most of the Asiatic chestnut plantations examined, *Cryptodiaporthe castanea* had the appearance of an active parasite, but was sometimes stopped completely by the callus formed around and over cankers during the summer months. Sometimes the killing was resumed the following year and the fungus invaded the tissue beyond the callous ring (Fig. 1, B). Although frost or other unfavorable factors may kill small twigs and allow the fungus to enter, there is no doubt that after it has entered the twigs it is able to invade and kill previously uninjured tissue.

A number of plantings of various strains of Asiatic chestnuts have been made on old abandoned fields, where the growing conditions are not yet suitable for hardwoods (2, 6) and, under such conditions, *Cryptodiaporthe castanea* may kill young seedlings to the ground. An attempt has been made to determine the relative effect of this fungus on vigorous and suppressed trees. Very few Asiatic chestnuts have been planted on sites that may be considered as offering optimum growing conditions. In some plantations the fungus severely attacks the most vigorous (dominant) as well as the weakest trees, but after analyzing the local conditions it was found that these strong-growing trees were not so vigorous as they should have

been and that the site in general was more or less unfavorable for Asiatic chestnuts. Near Mount Cuba, Delaware, a forest planting of a single strain of Chinese chestnut was made in the spring of 1926 on well-drained Chester loam. This planting is surrounded on 3 sides by native forest trees and the land planted to chestnut had formerly been in cultivation. The trees at the lower edge of the plantation have made excellent growth, averaging almost 3 feet increase in height a year. They are very straight and are surrounded by natural reproduction of tulip poplar, black birch, hickory, butternut, and black walnut. Trees in other parts of the plantation where the ground is covered with sod are shorter and more branched. Natural forest reproduction of hardwoods in this part of the site has failed to develop, although some seedlings have appeared and lived for a season or two before dying. A detailed comparison was made between an equal number of vigorous (dominant) chestnuts growing in association with natural reproduction of hardwoods and less vigorous (suppressed) chestnut trees growing in the open field. *Cryptodiaporthe castanea* was present on trees of both classes, but on the dominant ones it was confined almost entirely to the shaded lateral branches and many cankers had been healed over by callous formation. No appreciable amount of girdling was found on these trees and the organism was weakly parasitic. Numerous cankers were observed on the lateral branches and a few on the trunks of the suppressed trees. Some of these cankers had also been healed over by callus, but many were still active and some had girdled. It is believed that the disease does not seriously injure the trees except when trunks or major branches are girdled.

At Willets, N. C., a forest planting of the same strain of Chinese chestnut was made in the spring of 1926 on a north-facing cove site of well-drained Porter loam among small native hardwoods that had been thinned. The dominant chestnut trees in this plantation averaged from 2½ to more than 3 feet in height growth each year, while those suppressed by competition with other trees were stunted in comparison. A detailed examination of an equal number of dominant and suppressed chestnut trees in this plantation gave results similar to those obtained at Mount Cuba.

Cankers have been found on branches and trunks several inches in diameter, but no girdling has been observed on branches more than 1½ inches in diameter.

The imperfect stage of *Botryosphaeria ribis* has been collected on dead American chestnut twigs at several localities. It is also known to occur on various hosts over a considerable range. Stevens (17) stated that this currant cane-blight fungus is apparently fairly common on a variety of hosts, even in tropical and subtropical regions more than a thousand miles from the region where the currant is grown commercially, and suggested that the fungus was originally confined to native hosts of southern range. Smith (16) found that the host range of this fungus in nature includes at least 34 genera and 20 families of plants.

Very little is known about the potential damage that may be caused by the *Botryosphaeria* or *Diplodia* on Asiatic chestnuts, as they have been found on these hosts so infrequently that detailed studies under natural growing conditions have not been possible. Greenhouse inoculations indicate that they may be serious parasites, but conclusions based on artificial inoculations may be erroneous.

CONTROL

Investigations have shown that the disease induced by *Cryptodiaporthe castanea* is most prevalent and severe on weakened trees; hence the most important measure of control is to maintain high vigor of individual trees and of stands. Diller (6) recommended that forest plantings of Asiatic chestnuts be made on deep, well-drained, fertile soil suitable also for the establishment of volunteer hardwood seedlings such as tulip poplar, ash, hickory, and oak. It is believed that if the site on which chestnuts are to be planted is carefully selected so as to provide optimum growing conditions and the trees are carefully planted, the disease will not assume serious proportions. In orchard plantings the same care should be exercised in the selection of sites, but the possibility of fertilizing and cultivating the trees to maintain vigor may be taken into consideration.

Artificial inoculations and field examinations demonstrate that all three fungi may infect their hosts through wounds. Therefore, wounds should be avoided if possible, or if induced by pruning or in other ways, should be protected by a wound dressing such as asphaltum paint.

Since these fungi overwinter on cankers and dead twigs, all infected stems should be removed and burned during the early summer when the disease is easily recognizable. Stems should be pruned several inches below the infected area to make sure that none of the disease remains. Badly infected trees can be cut off near the ground and allowed to sprout from the roots, but should be uprooted if the infection extends to the base of the tree. These control measures are particularly emphasized for trees growing in nurseries and may possibly be supplemented by spraying.

Spraying nursery stock with a fungicide is now being tested, but as yet the results are too uncertain to permit recommendations. Means of spread of the fungi are not known.

SUMMARY

Asiatic chestnuts planted on poor sites have been found to be severely affected by canker and dieback diseases, while those planted on sites permitting optimum growth have not yet been found seriously affected. Several genera of fungi are represented by the fungi found associated with the dead twigs. *Cryptodiaporthe castanea, Botryosphaeria ribis chromogena,* and *Diplodia* sp. have been shown through inoculation experiments to be capable of invading living tissue and producing cankers and diebacks of Asiatic chestnut twigs.

Cryptodiaporthe castanea, the fungus most commonly found associated with the cankers of Asiatic chestnuts in the United States, has been found in 18 states and the District of Columbia. It has been present in 30- to 40-year-old Asiatic chestnut orchards for some time. It is not known whether this fungus is a native of this country. It was originally described on *Castanea sativa* in Europe and, if introduced, it has apparently been here for some time. It is sometimes damaging in nurseries and may cause the death of planted trees. It more frequently kills individual branches, thus decreasing growth and deforming the trees. The other two fungi have been collected only a few times and as yet are not known to be of great importance.

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ARTIFICIAL PRODUCTION OF "BLACKHEART" IN POTATO TUBERS

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It was first shown by Bartholomew (1) that blackheart could be artificially produced by subjecting the tubers to a temperature between 38° to 48° C. for 14–48 hours. Stewart and Mix (2) accidentally discovered that "by excluding the air from potatoes, blackheart may be produced at temperatures much lower than those employed by Bartholomew." They found that the length of time required to produce the disease was increased with an exposure of tubers to lower temperature, but it developed, even at 40° F. after 23–40 days, when the volume of air in the container was equal to the volume of the potatoes. It has been claimed that the injury resulting from poor aeration is due to a lack of oxygen and not to the accumulation of carbon dioxide given off during respiration. In this connection it is striking to note that Davis (3) was able to produce blackheart symptoms in potatoes, even when stored in CO_2-free air with an abundance of oxygen.

In connection with our work on the diastatic and oxidase activities of blackheart-diseased tubers (4), in the summer of 1935, we were able to produce typical blackheart symptoms by enclosing the tubers in hermetically-sealed jars at 30–56° C. for 6 days. But it was observed occasionally that the percentage of diseased tubers in potatoes sealed in 4-liter containers and heated at a given temperature for a fixed number of hours was extremely variable. This led us to believe that the condition of the tubers prior to heating with a view to produce blackheart was probably an important factor and that the variability in the results obtained was referable, in all probability, to our using a mixture of potatoes of various developmental stages. Moreover, in our work on the storage of potato tubers (5) it has been shown that the developmental stage of the tuber is a factor of considerable physiological significance. An experiment, therefore, was designed to test the relation between the developmental stage of the tubers and the percentage of blackheart induced at various temperatures.

During the growing season and the storage period of 1935–36, 6 samples were taken. Experiments were first performed on Dec. 1 and thereafter on Dec. 28, Jan. 21, March 10, May 20, and June 15. The work on the respiratory drifts and chemical composition (6, 7) showed that the tubers sampled on the above-mentioned dates belonged to the following more or less well-defined stages: adolescence, maturity and ripening (during the growing season) and early dormancy, middle dormancy, and late dormancy (during storage). Experiments were performed at 40.3°, 48.1°, 52.7° and 57.1° C. and the duration of heating varied from 48 to 105 hours. In all cases large-size tubers (var. Katua) were packed in sealed 4-liter containers, leaving a minimum of gas space. Relevant data are recorded in table 1.

TABLE 1.—*Relation between the developmental stage and the percentage production of blackheart at various temperatures*

Temp. °C.	Duration of heating (hours)	Developmental stage					
		Adolescence	Maturity	Ripening	Early-dormancy	Middle-dormancy	Late-dormancy
40.3 ± 0.3	48	No disease	No disease	No disease	No disease	20.2	22.7
"	70	"	"	"	"	41.7	41.2
"	90	"	"	"	"	46.2	37.2
48.1 ± 0.4	48	"	"	"	8.2	41.0	10.5
"	70	"	"	"	10.7	48.7	No disease
"	90	"	"	0.9	25.2	48.2	15.9
"	105	"	"	0.7	27.7	47.3	11.7
52.7 ± 0.5	48	"	"	1.2	27.0	47.2	No disease
"	70	"	0.1	1.7	30.1	50.2	50.2
"	90	"	0.4	2.0	35.2	54.7	10.7
"	105	"	No disease	2.1	35.1	56.2	15.7
57.1 ± 0.5	48	"	2.0	2.7	No disease	37.3	No disease
"	70	"	2.1	2.1	40.2	40.7	40.7
"	90	"	2.8	2.9	35.8	41.2	40.2
"	105	"	3.7	4.8	40.3	55.7	39.9

It is evident from the data that during adolescence, *i.e.*, when the tubers are very small and actively growing, even heating them at 57.1° C. for 105 hours could produce no visible signs of disease. On the contrary, during maturity, when the potatoes have attained more or less the maximum size, 0.1 per cent of the tubers were found to have contracted the disease when heated at 52.7° C. for 70 hours. During ripening, *i.e.*, when the potatoes had been in the soil for about 10 days after the stoppage of growth, 0.9 per cent of the tubers were found to de diseased in the lot heated at 48.1° C. for 90 hours. During early dormancy first signs of blackheart were observed in tubers maintained at 48.1° C. for 48 hours, whereas during middle dormancy 20.2 per cent of tubers were found to be diseased even when heated at 40.3° C. for 48 hours. Although no experiments were performed to test the assumption, it is very likely that the minimum temperature and duration for producing disease in potatoes in the middle-dormancy stage are much lower than 40.3° C. and 48 hours. The data obtained during the late-dormancy stage of the tubers show no general trend and are rather contradictory among themselves. For example, heating of potatoes at 52.7° C. for 70 hours produced 50.2 per cent diseased tubers, whereas the lot heated at the same temperature for 105 hours produced only 15.7 per cent diseased potatoes. This anomaly appears to be attributable to the fact that, during late dormancy, processes leading to sprouting had already commenced, and, although no sprouting tubers were included in the experiments, it is easy to imagine that the physiological and chemical changes inside the tubers preparatory to sprouting begin fairly in advance of the external appearance of sprouts, and were, on this account, probably included in the lots for experimentation undetected.

A consideration of the degree of susceptibility of adolescent and mature tubers to blackheart suggested the possibility of the size of the tuber being a factor in the incidence of blackheart. On May 20, 1936, some experiments

TABLE 2.—*Relation between the weight of potato tubers and their susceptibility to blackheart*

No. of tubers examined	Mean fresh weight	Percentage of diseased tubers
Variety Katua		
53	84.2	71.3
58	79.2	62.9
92	65.7	39.2
97	59.2	37.6
102	41.1	32.1
140	35.1	30.9
210	29.8	30.7
Variety Farrukhabad		
31	66.2	30.2
60	45.1	22.3
95	33.2	15.2
141	30.1	14.7
219	2.6	5.0
239	1.4	4.7

were performed on potatoes that, according to later respiration determinations and chemical analysis, were found to belong to the middle-dormancy stage. Two varieties of potatoes, Katua and Farrukhabad, were divided into a number of groups on the basis of their fresh weights. The potatoes belonging to each group were separately heated at 57.1° C. for 105 hours and the percentage of tubers showing signs of disease calculated. Relevant data are recorded in table 2. There is an indication that the bigger potatoes are more susceptible to blackheart than the smaller ones.

To sum up. Of potatoes of the various developmental stages those in the middle dormancy stage are most susceptible to blackheart. There is an indication that the smaller tubers are less likely to contract disease than the bigger ones.

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AGE OF SUSCEPTIBILITY OF RIBES PETIOLARE LEAVES TO INFECTION BY AECIOSPORES AND UREDIOSPORES OF CRONARTIUM RIBICOLA

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INTRODUCTION

Studies on the relative susceptibility of various native species of *Ribes* to infection by white pine blister rust (*Cronartium ribicola* Fischer) were among the first undertaken when the disease was discovered in eastern North America. In these early studies it soon became apparent that the age of the leaf at the time of exposure to infection is an important factor in its susceptibility.

Spaulding (8, p. 45) summarized his own observations and those of other eastern investigators up to 1919 and concluded that the age and relative maturity of the leaf are important factors in its susceptibility. The most favorable stage of growth, although there is considerable variation between species, seemed to be reached at about the time when the leaf attains full size, but has not become hardened and leathery as it does later.

In greenhouse inoculations of Pacific northwestern *Ribes* with urediospores of *Cronartium ribicola* and the native piñon blister rust (*C. occidentale* Hedgc., Beth., and Hunt), Hahn (2, p. 679) found that normal uredia were developed readily on fully developed leaves of *R. petiolare* Dougl. The uredia produced on immature leaves just below the growing shoot tips, however, were undersized and associated with necrotic flecks. This resistant type of infection was encountered also on the immature leaves of *R. viscosissimum* Pursh., *R. howellii* Greene, *R. laxiflorum* Pursh., *R. lacustre* (Pers.) Poir, and *R. sanguineum* Pursh. A somewhat different reaction was encountered when *R. triste* Pall. was inoculated. In preliminary tests Hahn (2, p. 679) found that leaves of this species showed a marked tendency to become infected for only a very limited period. Very young leaves produced necrotic flecks only; fully expanded leaves were susceptible but these leaves soon hardened and became apparently immune.

Inoculation results similar to those previously reported for *Ribes triste* were obtained by Hahn (3, p. 107) in a subsequent greenhouse test of several varieties of red and white garden currants with urediospores of the white pine and piñon blister rusts. He found that immature leaves did not become infected, nor did leaves that had commenced to harden.

Leaves of the susceptible *Ribes* used as "checks" in experiments with the blister-rust-immune Viking currant from Norway showed the same relationship between tissue development and rust infection (4, p. 9) as that encountered with leaves of *Ribes petiolare* and other Pacific northwestern *Ribes* previously studied (2, p. 679).

Lachmund (6) reported the results of studies conducted in the West in which *Ribes petiolare*, *R. inerme* Rydb., *R. viscosissimum*, and *R. lacustre*,

the principal *Ribes* associates of western white pine, *Pinus monticola* Dougl., were artificially inoculated in the field with aeciospores of *Cronartium ribicola*. These results indicated that the leaves were susceptible from the time they first emerge from the buds and that the period of highest susceptibility lay, in general, between the ages of 2 and 16 days (p. 113). As the leaves became older, their susceptibility to blister rust infection declined.

The results of the western study did not substantiate, in all details, those secured from the various studies conducted in the East. This lack of uniformity may be explained in part by the differences in methods employed. The eastern studies were conducted largely in the greenhouse with artificially propagated plants, and the inoculations were generally made with urediospores. The western study, on the contrary, was conducted on native plants growing out-of-doors, and aecial inoculum was used.

Lachmund (6, pp. 111–113) has shown that "the most favorable period for spread of the disease by aeciospores occurs when the period of maximum spore dispersal from a given center synchronizes with that in which the largest proportion of the leaves lying within the long-distance range of the spore showers are in their most susceptible stages." It is apparent, then, that the relative susceptibility of the *Ribes* leaves at time of inoculation is an important factor in their infection and, consequently, also, in the spread of the rust. In view of the somewhat contradictory results secured from eastern and western studies it was considered desirable to obtain additional information on the relationship between age of leaf and susceptibility. For this purpose the study herein reported was conducted at Moscow, Idaho, and methods more comparable to those of previous eastern studies were employed.

ORIGIN AND CARE OF TEST PLANTS

Ribes petiolare was used as the test plant in this study, since it is one of the most important *Ribes* species within the commercial range of western white pine (9), ranking fourth in abundance and first as introducer of the rust into a locality. The high susceptibility of this species has been demonstrated (2, 7) and data on the age of susceptibility of its leaves are available from both eastern (2) and western (6) studies.

The individual plants used in this study were collected near Clarkia, Idaho, in November, 1933. All were natural seedlings, 1 year old and from 3 to 5 inches in height. Fifty such plants were collected, potted in forest soil, and held over winter in the greenhouse[1] at Moscow, Idaho. At the beginning of actual experimental work the plants were stripped of their leaves and all lateral buds were removed. Of the 50 plants available, 30 individuals whose terminal buds burst at approximately the same time (February 10–11, 1934) were selected for the study.

RIBES LEAF DATA

The first series of leaves was tagged on February 15, 1934. These leaves had emerged from their buds within 5 days of each other and were mostly

[1] Greenhouse and laboratory space was provided through the courtesy of the School of Forestry, University of Idaho.

from 1 to 4 days of age, all having appeared between February 10 and 15. Every fourth day thereafter all new leaves were tagged with minute paper tags so as to distinguish between leaves of different 4-day age classes. Seventy-two days after the first leaves emerged from their buds the last series of leaves was tagged. A total of 18 consecutive 4-day age classes of leaves had thus been tagged; the leaves in the 69- to 72-day age class were those that first emerged from their buds. At least 8 of the age classes were represented on every plant; many individuals had nearly all age classes represented; and in every case, there was a difference of at least 53 days between the age of the youngest and oldest leaf on any plant.

On April 24 the last series of leaves was tagged and a leaf inventory was taken. The 30 plants were then divided into two groups, each of which had approximately the same number of leaves of all age classes represented. Between late evening, April 24, and early morning, April 25, a print was taken of each leaf by exposing a sheet of sensitized paper while closely appressed to the flattened leaf. These prints, when later planimetered, gave the area of leaf surface of different age classes at the time the last series of leaves was tagged.

INOCULATION OF RIBES

The aeciospores, with which one-half of the plants were inoculated, were collected on April 18, 1934, from blister-rust cankers on *Pinus monticola* in a natural infection area along Crystal Creek near Fernwood, Idaho. The urediospores, with which the remaining plants were inoculated, were collected from stock cultures maintained on *Ribes petiolare* in the greenhouse.

On the night of April 26, aqueous suspensions having a density of 100,000 spores per cubic centimeter were made up for each of the two spore types. Fifteen of the plants were then inoculated with the aeciospore suspension. Each leaf was inoculated individually, while shielded from the remaining leaves, by giving the lower surface 2 blasts from a De Vilbiss atomizer held at a constant distance from the leaf. This distance was sufficiently great to cause the spore suspension to reach the leaf in the form of a dense vapor rather than as a definite stream, thus insuring uniformity of application per unit area irrespective of size of leaf. All leaves on the other 15 plants were inoculated in a similar fashion, but with the urediospore suspension. The application was checked by spraying the spore suspensions onto glass slides in the same manner in which the leaves had been sprayed. An examination of these slides showed that a comparable number of each type of spore had been applied per unit of leaf area. A bell jar was placed over each individual plant immediately after inoculation and left there for 36 hours to provide more favorable infection conditions. After removal of the bell jars the inoculated plants remained in the greenhouse until the study was completed.

RESULTS

On May 7 and 8 the leaves were stripped from the bushes and dried in a plant press. The length of time since inoculation (11 to 12 days) was con-

sidered sufficient to permit the vast majority, if not all, leaf flecks to make their appearance, Spaulding (8, p. 40) having shown from greenhouse tests that, in 63 per cent of the cases, 12 days was ample time for infections to develop into mature uredia and that all infections developed mature uredia within 17 days. Observation showed that none of the infections on leaves of the test plants had developed far enough to produce urediospores that might have intensified the rust.

After the leaves were dried the flecks indicative of infection by *Cronartium ribicola* (1, p. 634; 5, p. 874; 8, pp. 53–54, Pl. VI, Fig. 2) were carefully counted for each of the 18 leaf age classes represented. For purposes of comparison the number of leaf flecks in each age class was converted to a flecks-per-10-square-centimeter basis. Fertile uredia may not have developed eventually from every leaf fleck. From previous infection studies (6, 7), however, involving thousands of infected *Ribes petiolare* leaves, it has been observed that leaf flecks develop fertile uredia with equal facility when originating from either aecial or uredial infections. In determining the relative susceptibility of different aged *R. petiolare* leaves the relative number of leaf flecks developed is, therefore, a reliable criterion.

All inoculated plants and inoculated leaves of all ages became infected, although every leaf inoculated did not become infected. Table 1 shows the final results of this study for both the aeciospore and the urediospore inoculations. With both spore types the highest susceptibility (as judged both by percentage of leaves infected and by number of leaf flecks per unit area) was found on leaves in the younger age classes. A decrease in susceptibility was found to accompany increased leaf age irrespective of spore type used in making the inoculation. Results of the aeciospore test showed leaves up to and including those in the 9 to 12-day age class to be decidedly more susceptible than older leaves. With urediospores, however, this high susceptibility definitely extended to leaves in the 13- to 16-day age class and, to a lesser extent, leaves even as old as those in the 21- to 24-day age class exhibited fairly high susceptibility.

It is interesting to compare these ages of highest susceptibility with leaf development. By combining all leaves of a given age class and computing their average size, it was found that the *Ribes petiolare* leaves apparently required about 3 weeks to reach size-maturity after emerging from the buds. It must be realized, however, that there is great individual variation in leaf size at maturity and that this individual variation accounts for the differences between sizes of leaves in the aeciospores and urediospore tests, as are shown by age classes in table 1. The data do indicate, nevertheless, that the *Ribes* leaves were in their most susceptible state during the first few days after emergence and that they remained highly susceptible until about the time of size-maturity, after which their susceptibility declined. This conclusion is in close agreement with that reached by Lachmund from field studies previously reported (6, p. 113).

Extremely young leaves became infected in both the aeciospore and the urediospore tests. Unfortunately, there was at least a 4-day range in each

TABLE 1.—*Age of susceptibility of Ribes petiolare leaves to infection by Cronartium ribicola as determined by greenhouse inoculations with aeciospores and urediospores*

Leaf[a] age class	Aeciospore inoculation							Urediospore inoculation						
	Total plants	Number of leaves		Leaf area[a]		Rust flecks	Flecks per 10 sq. cm.	Total plants	Number of leaves		Leaf area[a]		Rust flecks	Flecks per 10 sq. cm.
		Total	Infected	Total	Average				Total	Infected	Total	Average		
(*Days*)	*No.*			*Sq. cm.*	*Sq. cm.*	*No.*	*No.*	*No.*			*Sq. cm.*	*Sq. cm.*	*No.*	*No.*
1– 4	11	19	17	39	2	622	158	13	18	15	36	2	457	127
5– 8	13	16	16	164	10	2244	137	11	13	13	103	8	1844	179
9–12	13	17	17	302	18	3037	101	9	10	10	168	17	2522	150
13–16	11	13	13	356	27	1656	47	12	14	14	320	23	3857	120
17–20	11	12	12	349	29	1297	37	11	11	11	352	32	3379	96
21–24	9	11	11	281	26	917	33	9	11	11	380	35	3713	98
25–28	9	9	9	243	27	449	18	5	5	5	151	30	798	53
29–32	7	7	6	198	28	441	22	10	10	10	301	30	1386	46
33–36	8	9	8	222	25	623	28	10	10	9	300	30	868	29
37–40	7	7	7	196	28	329	17	9	10	9	325	33	892	27
41–44	9	9	9	213	24	511	24	12	12	10	294	25	936	32
45–48	13	14	13	326	23	489	15	9	12	9	296	25	785	27
49–52	11	15	12	410	27	567	14	11	13	11	296	23	280	9
53–56	10	14	10	229	16	294	13	8	16	14	371	23	512	14
57–60	10	13	9	262	20	214	8	10	16	8	247	15	284	12
61–64	9	11	7	331	30	326	10	6	8	3	210	26	26	1
65–68	12	16	10	575	36	583	10	13	18	10	458	25	266	6
69–72	11	14	8	570	41	272	5	12	18	14	622	35	254	4

[a] Age and area at time of making leaf prints (April 24–25). Since the inoculations were not made until April 26, the leaves, especially those in the younger age classes, were slightly larger than is shown. Similarly, the 1- to 4-day age class includes leaves actually 2 to 6 days of age, and the leaves in all other age classes are 2 days older than the designated extremes.

age class, making it impossible to state definitely that the leaves were susceptible immediately upon emergence from the buds. After exhibiting comparatively high susceptibility to *Cronartium ribicola* infection while very young, the leaves of *Ribes petiolare* became less and less susceptible (or more and more resistant) as they became older. This was true irrespective of type of spore used as inoculum. Although the trend was somewhat erratic because of the small basis in certain age classes, it was definite and was maintained until the leaves were at least 71 to 74 days old (69- to 72-day age class). Under greenhouse conditions, even the oldest leaves tested did not present the hardened, leathery appearance overmature leaves exhibit as they develop under field conditions. Since these leaves were relatively low in susceptibility, it would seem that something in addition to leaf succulence is responsible for the differences in susceptibility found between leaves of various ages.

Table 1 shows that both aeciospores and urediospores of *Cronartium ribicola* are capable of causing infection on *Ribes petiolare* leaves of all ages, from those but 2 to 6 days old to those 71 to 74 days old. No attempt should be made, however, to draw conclusions from this table as to the comparative infecting ability of these 2 spore types. The factor of spore viability was not sufficiently well controlled to permit such a comparison to be drawn.

SUMMARY

The leaves produced on 30 *Ribes petiolare* seedlings, maintained in the greenhouse at Moscow, Idaho, were tagged at 4-day intervals until leaves in all age classes up to those in the 69- to 72-day age class, inclusive, were represented. The plants were then divided into 2 equal groups and the area of each leaf determined. On April 26, 1934, 2 days after making the area determinations, one group of these plants was inoculated with an aqueous suspension of *Cronartium ribicola* aeciospores and the other group with a similar suspension of urediospores of the same heteroecious rust. After an incubation period of 11 to 12 days the leaves were stripped from the plants and the flecks indicative of infection were counted.

All plants and leaves of all ages became infected. The relative susceptibility of leaves of different ages was determined by comparing the number of flecks per unit area at the approximate time of inoculation. The younger leaves were found to be the most susceptible to infection from both spore types. Leaf susceptibility remained high until about the time of size-maturity (approximately 3 weeks after emerging from the buds). After reaching size-maturity an increase in leaf age was accompanied by a decrease in susceptibility.

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SOME ASPECTS OF THE CONTROL OF SPIKE DISEASE IN SANDALWOOD

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INTRODUCTION

Spike disease in sandal represents a diseased condition of the leaves and twigs. It manifests itself in an abnormal vegetative activity of the plant and is diagnosed by a characteristic reduction in leaf size, and by an extremely striking disposition of the internodes at regular but short intervals. With this enhanced vegetative growth as evinced by excessive branching of the diseased parts, the reproductive phase is entirely inhibited in them. Even the few fruits that may be formed are incapable of germination. Moreover, the diseased plants eventually die. In view of this interesting feature about this malady, which appears to threaten the existence of this economically important species—a monopoly of South India—some Governments have spent time and money in discovering a proper solution for it. An attempt is made here to review our present knowledge in controlling this pest.

The necessity for controlling the disease arises from the fact that it is highly infectious in character, as shown by Coleman (7) and by Sreenivasaya and Naidu (14). In other words, the disease could be reproduced at will artificially. In the absence of bacteria, etc., and in the light of evidence presented by Narasimhan (11), it is considered to be virus in nature. Though its origin remains obscure, it has made considerable advance into many of the sandal forests within the last 40 years. As sandal is one of the important revenue sources for Mysore, Coorg, and Madras, the advent of spike disease has been a curse. The spread has been in a southeasterly direction, causing much

havoc to the several localities. The rate of attack has varied enormously. As a rule it spreads slowly and steadily, but in some years, the attack has assumed highly virulent and epidemic proportions. There are areas where the infection exists in such virulent form that it had proved a hopeless problem for the forest departments. In a similar way, infectivity varies in individual plants. While it is normal to see a plant with 1 or 2 shoots primarily affected, several instances have been observed where the crown is diseased all over, simultaneously. Thus, there is no knowing when an area is ready to be infected and, if infected, whether the attack will be a mild one or will assume epidemic proportions. All these observations demand a prompt action for controlling spike disease, if sandal is still to be the monopoly of the Deccan plateau.

ELIMINATION OF FACTORS CONDUCIVE TO THE SPREAD OF INFECTION

Although spike disease is ultimately fatal, the affected tree continues to put forth its characteristically reduced leaves for a maximum period of 3 years, after which it dies. Therefore, even one infected plant is a veritable source of infection to its healthy neighbor. The mechanism of this transference of disease is varied, apart from the fact that such centers of infection are varied also. Unless, therefore, the radiating points of infection are rooted out, the problem of controlling spike disease is not solved. In what manner can this be done at a cost that will admit of ready adoption, in addition to its being efficient also, will be treated here.

Through the Removal of the Diseased Plant Itself. The suggestions of Butler (5) to create a belt of 100 feet around an infected patch was found to check the spread of disease only for a time. This is feasible primarily in incipient stages of attack and also when small patches are to be treated. On the other hand, mechanical removal and complete destruction of spiked plants has involved a large expenditure without corresponding quickness in effect. But where this procedure has been adopted with promptitude, effective check on the rate of infectivity has been recorded. This is the real clue to the problem on hand. The practice of awarding one anna for every spiked plant pulled out, once cost the Madras Government an enormous sum of money. The difficulty here was one of identifying whether a plant actually was affected or not; obviously, even healthy ones were removed. All the same, this was the only recognized method. If, therefore, the diseased plant can be effectively eliminated, the problem is somewhat easier to deal with.

Where this plant removal has not effectively been carried out, troubles have arisen. For instance, sandal is known to produce coppice shoots, if the trunk is lopped. These shoots are more numerous but concentrated, with reference to the active principle, when the spiked plant is cut at its bole. Another feature of sandal is its readiness to produce root suckers, which have their origin in the cut portions of roots. Such plants are bound to arise, since the removal of roots by mechanical means is never complete. It is known also that roots of diseased trees carry the infective principle in

them. Spiked plants also produce root suckers which are diseased and hence contribute to further spread of infection. Thus, there is provided a ready means of establishing the disease. The question of root suckers in sandal is something extraordinary. Thus, in Coorg, the writer came across 2 sandal plants 1 on either side of a road, differing from each other in height. On examination it was found that the smaller plant was a root sucker from the bigger one. Again, in the course of some experiments on artificial transmission of spike in another locality, certain uninoculated plants revealed symptoms of spike, but were later on discovered to be root suckers from the treated ones. These raise problems of great significance as to whether, during the incubation period, the roots are primarily infective, or whether the infective principle is carried to the root ends. In any event, the elimination of such plants is the most immediate problem. It is evident from this, that an incomplete removal of the diseased plant does not prevent the spread of spike.

It was recorded fairly early that sandal was a root parasite. But we owe it to the late Dr. Barber (2) who elaborated it in his extensive researches on sandal in relation to its nutritive requirements. He also showed that sandal grew best in the presence of host plants for a period of at least 5 years (3), a fact that has since been corroborated by Sreenivasa Rau (13). But Sir Dietrich Brandis' (4) question whether sandal does not derive a part, though little, of its nutrition from the soil also, has not adequately been answered. It is probable that sandal roots derive some nutrition through the root caps, in the absence of root hairs. In view of the capacity of sandal to form haustoria on its own roots, it is of significance to find that healthy plants also can be infected possibly by parasitizing the roots of a spiked plant. Such a possibility was indicated by Coleman (8), although our own tests in the Indian Institute of Science, Bangalore, under artificial conditions, have thus far been unsuccessful in this direction. This is one more reason why the diseased plant should be entirely eliminated as early as possible.

It has often been said that spike is a purely physiological manifestation arising out of unfavorable environmental conditions. By a comparative study of infected and healthy areas, Varadaraja Iyengar and Rangaswami (20) showed that the death rate of sandal in the former was abnormally high. The further fact that localities far distant from one another also are affected indicates the infectious nature of the disease. Great significance also is attached to the beneficial results noticed in areas from which spiked plants have been removed. A series of investigations on the control of spiked trees by the above authors (20) has led to the conclusion that arsenicals are the best plant poisons for the purpose. Others also may act equally well, but their high cost, for example, that of sodium chlorate for application in greater concentrations, is not commensurate with the results secured. The success of the new procedure has been well established. Thus, several localities, where spike has been reported in incipient stages, have been saved from the invasion of this pest. In other words, but for this simple technique and

process developed by them, many of the sandal areas would have been doomed. Sodium arsenite is reasonably cheap and is now available on the market in a highly improved condition evolved by the writer.

The problem now resolves itself into a control of areas both mildly and virulently affected. In the former case, a prompt action based on the above technique has proved successful. Where healthy trees are few compared with diseased ones, or where the rate of spread of spike is epidemic in character, the problem is more complicated. Although it may be argued that the disease itself may exterminate the species within a few years, experience does not corroborate this view. There are several blocks where sandal is prolific and the disease also is fairly active. There is no record of any area having been entirely wiped out in this manner. In such cases bold action is essential. It is true that the cost of treatment in such localities is high. But the advantage lies in eradicating the infection center at a shorter period than can be imagined. It is, therefore, necessary that a concerted attack in controlling the spread of disease should be made, if the monopoly of sandal is to be retained. The cost involved in this is really worth while. A special staff may have to be temporarily appointed for this purpose. It may be remarked here that on an average 150 trees can be treated with one gallon of the solution. Hence an enumeration must first be made to compute the necessary amount of money required. It is urged that the Governments concerned will take up this question at once.

Without going into greater details, it may be remarked that our tests with the poison on diseased plants have proved effective and innocuous. The effect of the poison is visible within a week to a fortnight, depending on the season of application. Where the first dose has failed—this may be due to want of care in treating—a fresh application should be made. In some cases of virulent infection, a different mode of treatment will be better. The coppiced stumps respond well to the poison when applied on the cut surface. In any event, it was found through experiments that both the heartwood and the oil obtained therefrom contained essentially no arsenic (17). In other words, the therapeutic value of these commodities does not appear to have been impaired by this method to any extent whatever. It is also on record that by this treatment, root suckers of diseased plants have been killed outright. It is claimed, therefore, that the above process is the best for controlling the disease in the quickest possible time.

(b) *Through the Removal of Possible Alternate Hosts of Sandal Spike.* It is well known that there are several species occurring in the forest side by side with sandal, and many of these are found to exhibit spike-like symptoms, the nature and cause of which are yet obscure. *Zizyphus oenoplia*, *Dodonea viscosa*, *Vinca rosea* and a few others are the most important of these, although the total number of such manifestations is nearly 40. Many more have to be included in this list. It is of significance, however, to find that spike in *V. rosea* is almost identical with that in sandal as shown by Narasimhan (11) and by Varadaraja Iyengar (18). Whether all these were

attacked simultaneously with sandal, is too much to say. But one thing is certain; that these diseased plants are to be seen in localities where the sandal is not known to grow. Such definite instances have been recorded by Barber and also confirmed by the writer. More cannot be said on the identity of these. Even though the symptoms do not closely agree in all respects, different species may react in varying manner to the active principle. The possible relation of these acting as alternate hosts to sandal spike cannot, therefore, be ignored. Our own evidence on it from artificial-transmission studies is weak. That insects may be better fitted to effect such transferences is well recognized. In order therefore to prevent the spread of infection in sandal, such diseased plants must be eliminated. The author has tried sodium chlorate for the purpose. But in those cases where the plants are big, like those of *Z. oenoplia*, preparations containing sodium arsenite have been successfully applied, as with spiked sandal. It is, however, recognized that more attention should be paid to this aspect of the problem. Besides these, the possibility of other virus infections of plants serving as alternate hosts to sandal spike, has to be eliminated, but in the writer's opinion, it is only of secondary importance.

(c) *Through Factors Relating to the Carrier of Infection.* A preliminary study on this point was made by Hearsey (10) and reported in 1917, in which he could not incriminate any particular species with the transmission of spike. In a similar way, Chatterjee (6) failed in his attempt, although he has contributed a great deal on the insect relations of sandal. But Dover announced (1) that one of the sap-sucking type of insects (*Moonia albimaculata*) was responsible for transferring the disease to a healthy plant in pots almost at the close of the spike investigation scheme. Much controversy arose over his discovery. His evidence was based on the presence of X-bodies in such leaves as per technique of Narasimhan and the nitrogen content of the same specimens. In dealing with these tests one may point out that nitrogen values are never reliable for such purposes, and that he should have secured data for calcium and the calcium/nitrogen ratio, the surest index. The data for biometric measurements, referred to in a later publication (9), have been critically examined by the writer elsewhere (19). Suffice it to point out here that the writer's own measurements on them corresponded with those for healthy specimens. A similar examination served to eliminate Chatterjee's claims of a beetle as a possible carrier (19). It is indeed doubtful whether Sreenivasaya's technique of lopping these plants is a reliable one, and will be referred to later.

The real transmission of spike through insects was discovered by Rangaswami and Sreenivasaya (12) who showed that under cage conditions, healthy plants in pots exhibited symptoms of disease when a mixed fauna gathered from the forests was dumped into it. They claimed that the night collections were those responsible for this transference. Further experiments with individual groups and species have not, so far, been successful. The mass-feeding series was repeated with success (15).

As long as our knowledge is obscure on the insect to be incriminated with the transmission of disease, attempts to control the carrier of infection will be futile—nay, even unwise. It is known that treatments differ depending upon the nature of the fauna involved. Two methods of control are recognized, biological and biochemical. In the former case, the necessary parasite is introduced to inhibit the development of the insect vector. In the latter, both diseased and susceptible plants are sprayed with chemicals, the nature of which differs with the type of insect. In either case, the writer agrees with Dr. Butler (private communication) that control of spike disease is a very costly process.

IDENTIFICATION OF DISEASE

Whatever treatment may be recommended for spike, there is one aspect that has not been adequately recognized. It relates to the identification of the potential source of infection. In other words, the symptoms generally described and understood are not sufficiently indicative of the distinction between a healthy and spiked plant. While it is normally easy to identify affected ones, many instances have been brought to the writer's notice that have led to some confusion, even among experienced workers. This is complicated by the fact that growth in sandal is conditioned to a great degree by its parasitic habit and hence, by the nature of the host plant associated with it. In several areas, healthy trees have been noticed with foliage apparently resembling spiked ones. Some of these have been referred to elsewhere by the writer (19). In order to eliminate the difficulties normally encountered, a simple technique of a rough and ready nature was evolved by the author. This has stood the test quite well in the field, being based on leaf and internodal measurements. A more accurate and definite index was discovered by the author on the physiological data secured from different areas for a large number of specimens. Thus, the ratio Ca/N is the safest for the purpose (19); in fact, failure to obtain such data for those cases wherein transmission of spike through insects has been claimed has resulted in some confusion.

A point of equal importance is that of disease masking. It is claimed by Sreenivasaya that certain apparently healthy plants develop symptoms of spike when they are lopped, *i.e.*, deprived of their crown. The basis for this arises out of some experiment in which artificially infected plants were found to manifest the disease on being lopped. No evidence is available as to the particular stage following inoculation when this is reproducible. Evidence also is lacking on the possible infective nature of such specimens by grafting and other tests. There appears to be some confusion on this point. One ought to distinguish between incubation period and disease-masking stage. In fact, scores of experiments carried out at Noganoor revealed the astounding feature that certain plants did not exhibit spike symptoms even after 380 days. It is therefore naturally felt that lopping is not a wise technique. Moreover in some of the earlier experiments on controlling spread of spike,

only those trees that manifested the disease were removed with the result that the area was later completely free from infection for several years. This aspect of the problem is quite complex, requiring elucidation of several points based on extensive experimentation.

FACTORS RELATING TO SUSCEPTIBILITY AND RESISTANCE TO SPIKE INFECTION

It was remarked earlier that the parasitism of sandal is a feature to reckon with. It is, therefore, natural to expect that the character of the associated host plant should primarily contribute to the weakness or otherwise of the parasite. In fact, the growth of sandal varies with different hosts. Leguminous plants, such as *Acacias*, pongamia, etc., help to a more rapid growth of sandal. Even among the legumes, there appears to be a difference in the supply of food materials as evinced by the growth and development of the parasite. These host plants aid sandal by a large supply of nitrogen in addition to minerals. Moreover, the rate of attack by sandal roots varies with diverse species. In the field, one encounters innumerable haustorial attachments on the roots of lantana, pongamia, and others, while many other species are not so heavily invaded. What conditions determine the life and duration of haustoria are still obscure.

With reference to spike disease, some of these host plants act adversely, by weakening sandal. Thus, the incubation period is considerably diminished when sandal growing in association with *Acacia* sp., etc., is grafted with diseased tissues. Only very few species really favor it with a capacity to resist disease, as judged by the incubation time. Most of these experiments have been carried out in pots, and demand considerable care in interpreting the same, when a perennial such as sandal is concerned. Until now no one single species can be claimed to have offered complete immunity against infection for sandal.

But, in a general way, it may be said that in most of the areas where spike has had a sway, lantana is predominantly present. Even though stray plots and patches can be discovered with spike and no lantana, the more common observation relates to a higher incidence of disease in lantana-infested areas. The rôle of lantana in relation to sandal spike already has been referred to by the author (16). The more direct test should confine itself to elimination of this weed and then compute the rate of spread with adequate controls. Experiments on the removal of the exotic through chemical treatments have been successfully tried.[1] This process is more economical, too. Further reference to this problem will be made elsewhere.

Although no correlation has been possible between physiological factors and spike incidence, there is one observation that deserves close examination. This relates to the fact that a predisposed tree or one that is likely to get infected in the coming season, produces an abnormally large quantity of flowers. The composition of the leaves at this stage is marked by a high lime

[1] Varadaraja Iyengar, A. V. Thesis, degree of Doctor of Science, Madras Univ. 1934.

content and a significantly low value for nitrogen. These observations are in striking contrast with what has been recorded by the writer in the case of diseased ones in which the reproductive phase is completely inhibited.

CONCLUSION

In the résumé presented here, it has been indicated that the infectious nature of spike necessitates prompt action in eliminating all possible factors that contribute to the spread of the disease in the forest. Since there is no certainty as to whether the attack may be mild or virulent, or as to the area that may next be affected, this problem assumes greater importance. The most serious item for attention is the diseased plant itself. The significance of this aspect has been strikingly brought out by the fact that this is the center of infection, no matter what the method of transmission, whether through haustoria, rootsuckers, or insects. Therefore, attention has been drawn to its economically accomplished elimination through application of toxic chemicals, whereby alone, the spread of disease has been and can be considerably checked. This procedure is now adopted in Madras and Coorg. A few areas with highly virulent stage of spike must be dealt with in the above manner, in order to keep the spread of infection under control.

Our knowledge of the carrier of infection is still obscure. Even here, removal of the diseased plant simplifies the problem. The possibility of the carrier of infection transmitting the disease from other alternate hosts of spike has not been lost sight of, but seems to be of less importance.

Sufficient attention has been drawn to the necessity of proper diagnosis of the disease under field conditions and the phenomenon of masking of spike. Much yet remains to be done on the factors of susceptibility and resistance of this plant parasite to the invasion of one of its most serious diseases. A good deal of improvement on the growth of sandal can be realized through eradication of lantana, a plant that has slowly permeated even some of the dense forests of the Deccan plateau.

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SOME EFFECTS OF RUST INFECTION ON THE DRY WEIGHT OF HOST TISSUES[1]

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INTRODUCTION

In the literature dealing with the effect of rust fungi on their hosts, no satisfactory data have been found on the effect of rust infection on the dry weight of infected tissues. Where hypertrophy of the host occurs in response to rust infection, a local increase in dry weight is to be inferred; in the absence of hypertrophy, the weight of the mycelium might be expected to cause an increase in dry weight in such compatible host-parasite relations as exist in most rust infections (1, 2). The previous observation of the senior writer that rust infection increases the dry weight of clover leaflets floated on sucrose solution in the dark (7) is not a reliable index to the effect of rust on leaves attached to the growing plant.

The present study, principally with the uredial stages of *Uromyces phaseoli* (Pers.) Wint. on bean, *Phaseolus vulgaris* L., and *Puccinia helianthi* Schw. on sunflower, *Helianthus annuus* L.—two rusts that affect only or principally the leaves of their hosts, and that cause no obvious hypertrophy —is an attempt to analyze the nature of the effect of these fungi on the dry weights of their hosts.

The term "increase," as here used, means the greater dry weight, or green weight, of infected plant tissues in comparison with control noninfected tissues of similar area and age, grown under the same environment, and harvested simultaneously; and "decrease" means the lowered weight of tissues due to rust infection.

[1] The assistance of nontechnical employees of the Works Progress Administration is acknowledged.

THE DRY WEIGHT OF THE RUST-INFECTED REGIONS OF LEAVES

The effect of rust infection on the dry weight of leaf tissues of 11 hosts was studied by comparing the dry weight of discs of rust-infected tissue with the weight of similarly located discs of healthy tissue from the opposite halves of the same leaves. The discs of the first 3 collections listed in table 1 were 6.7 mm. in diameter, the remainder were 3 mm. in diameter. The use of smaller discs made possible a better separation of infected and non-infected tissues. From field collections it was difficult to secure closely comparable paired units in which all the infected samples were uniformly infected and all the control samples were entirely free of infection; therefore, an estimate is recorded of the percentage of the area infected in the different samples. The samples were dried to constant weight at 70° C. and weighed. The difference in weight between the paired rusted and control samples was expressed as a percentage of the weight of the controls.

In table 1 is shown the effect of various rusts on the dry weight of infected leaf tissues of their respective hosts, as measured by the above method. With the exception of the last 2 collections that were from greenhouses, all material listed was collected in the field, and in all cases the duration of the infection was not known. The material was collected during the day and sample discs were cut several hours later. As will be shown later (Table 4), the time of day of collecting samples may be an important factor in the results obtained. For each dry-weight determination there were 3 to 15 discs in each member of a paired sample, and 3 to 17 pairs of samples for each value listed.

The accuracy of this method of studying dry-weight changes may be illustrated by the later results with beans. With 60 pairs of leaf samples of five 3-mm. discs each (Table 4), the average deviation of a single determination of dry weight of healthy tissue per unit area from the mean of 10 determinations was 11.9 per cent of the mean value, and the average deviation of a single determination of dry-weight change attributable to rust infection from the mean of 10 determinations was 28.2 per cent of the mean value.

With the exception of *Puccinia canaliculata* on *Xanthium* sp., which was in the aecial stage, all rust collections listed were in the uredial stage, and in no case, with the exception of *Xanthium*, was there any apparent hypertrophy of the host tissue because of rust infection.

The results given in table 1 indicate that rust-infected leaf tissues generally contain considerably more dry matter than comparable healthy tissues. The tabulated results with *Melampsora* on *Populus* are an exception to this rule, but these results may be atypical.

THE DRY WEIGHT OF ENTIRE INFECTED SUNFLOWER LEAVES

The dry weights of infected and noninfected paired primary leaves on the same plant, and the dry weights of leaves of infected and noninfected paired plants, and paired pots of plants were compared (Table 2), using

TABLE 1.—*The effect of rust infection on the local dry weight of leaf tissue of different hosts*

Date	Pathogen and host	Number of paired samples	Estimated percentage area of samples visibly infected with rust		Dry weight of samples, per square decimeter		Percentage difference in dry weight of rusted tissue compared with healthy
			Control	Rusted	Control	Rusted	
			Per cent	*Per cent*	*Mg.*	*Mg.*	*Per cent*
Sept. 12, 1934	*Uromyces betae* on *Beta vulgaris*	5	5	40	955	1219	+ 27.7
" 15, "	*Tranzschelia punctata* on *Prunus domestica*	3	5	15	840	853	+ 1.5
" 17, "	*Melampsora* sp. on *Populus* sp.	5	10	30	1264	1225	− 3.1
Oct. 1, 1934	*Uromyces phaseoli* on *Phaseolus vulgaris*	5	5	40	815	968	+ 18.8
" 11, "	*Puccinia canaliculata* on *Cyperus esculentus*	5	0	80	519	1090	+110.0
" 11, "	*Puccinia canaliculata* on *Xanthium* sp.	5	0	100	1450	2488	+ 71.5
" 11, "	*Puccinia polygoni-amphibii* on *Polygonum acre*	5	5	50	1058	1144	+ 8.1
Nov. 26, 1937	*Puccinia iridis* on *Iris* sp.	5	5	80	1180	1498	+ 26.9
Dec. 3, "	*Puccinia antirrhini* on *Antirrhinum majus*	9	5	100	1346	1809	+ 34.4
" 9, "	*Phragmidium* sp. on *Rosa* sp.	17	2	40	504	534	+ 10.6
	Puccinia helianthi on *Helianthus annuus*	12	0	70	227	273	+ 20.5

greenhouse-grown sunflowers. The noninfected leaf of the pair on a single plant was protected during inoculation by a previous spraying with 1 per cent lime-sulphur + 0.1 per cent sodium oleyl sulphate, or by covering with a glycine bag. The fungicide was found to increase the dry weight of the treated leaves by about 2 per cent, an amount so small that it had little effect on the weights being measured. In all cases where leaves on one set of plants were compared with check leaves on another set, the pairing was done before inoculation.

TABLE 2.—*Effect of rust infection on the dry weight of sunflower leaves 1937*

Units compared	Date of test	Infection period	Paired samples	Increase in dry weight of rusted leaves over paired control leaves[a]
		Days	*No. of*	*Per cent*
Twin leaves	March 19[b]	15	13	28.8 ± 6.5
	" 30[b]	10	13	11.3 ± 2.3
	" 30[b]	14	14	21.7 ± 3.2
	Nov. 27	12	16	12.3 ± 3.4
	" 27	26	5	32.1 ± 9.7[c]
Paired pots	April 12	11	5	10.9 ± 2.4
	" 23	15	10	10.4 ± 6.9
Paired plants	Aug. 24	15	12	17.9 ± 7.6
	" 24	28	8	27.9 ± 17.8
	Oct. 19	11	10	15.2 ± 7.3
	" 19	20	10	13.5 ± 9.7
	" 19	28	10	2.0 ± 4.2[c]
	Nov. 12	14	9	7.3 ± 9.7
	" 12	21	8	45.9 ± 19.1

[a] Mean and standard error of mean.

[b] Control leaves were protected by spraying with 1 per cent lime sulphur + 0.1 per cent sodium oleyl sulphate. In all other cases controls were protected by means of glycine bags, if necessary.

[c] Rusted leaves dying or dead.

Potted plants were inoculated by dusting spores of *Puccinia helianthi* on the upper surface of the leaves, atomizing them with water, and holding them overnight in moist chambers. This method does not produce such heavy infection as do inoculations on the lower leaf surface, but the infection was more uniform.

The severity of infection was not measured in most tests, but, in the test of November 12, the 44 leaves varied from 10.3 to 25.2 sq. cm. in area and the number of primary pustules per square centimeter varied from 25 to 87. This is heavier than the infection in most tests.

This method of making comparisons between opposite twin leaves, one of which is infected and the other not, is believed to be very accurate because twin sunflower leaves and the twin leaves of many other plants (4) are very closely comparable. With 22 random pairs of primary sunflower leaves from plants of the same age, the average deviation of a single leaf from the mean of a pair of leaves was only 3.6 per cent of the mean value for area and 3.9 per cent for dry weight, while for the same leaves the average devia-

tion of a single leaf from the mean of all leaves was 19.7 per cent for area and 24.5 per cent for dry weight. A part of the measured deviation in the paired samples was probably caused by errors in the rapid planimeter measurements of leaf area and the weighing of the leaves to only the nearest milligram.

The results of 14 tests with a total of 143 paired samples (Table 2), show that rust-infected leaves were always greater in dry weight than paired control leaves, the measured difference varying from 2 to 46 per cent. The reason for the great range of difference is partly the difference in infection period. In 4 cases (March 30, August 24, November 12, and November 27) 2 tests with different infection periods were started on the same day, and in each case the difference in dry weight was much greater with the longer infection period. The test of October 19, however, is an exception, for here the difference due to rust was less with longer infection periods. In this test, however, paired plants were compared and error is much greater than with paired leaves. It would seem quite possible that the effect of rust infection might be entirely different when only 1 leaf of a pair is infected than when both are infected. The present results, though the data are inadequate, would indicate, however, that the effect of rust on dry weight is similar whether comparisons are made between 2 leaves of a single pair, between paired plants, or between paired pots of plants.

Rust-infected leaves were also greater in green weight, and in percentage dry weight of the original green weight, than healthy leaves. In 3 tests (the same as the first 3 tests of table 2) rusted leaves were greater in green weight by 8.5 ± 5.3 per cent, 6.1 ± 2.7 per cent, and 11.9 ± 2.3 per cent, respectively, than healthy control leaves. In these same tests the percentage dry matter of healthy leaves was 13.2, 14.9, and 12.9, respectively; and of rusted leaves 15.5, 15.7, and 13.9. The percentage dry matter in the rusted leaves was greater by 14.7 ± 2.45 per cent in the 3 tests comprising 40 pairs of determinations.

EFFECT OF RUST INFECTION ON THE GROWTH OF SUNFLOWER AND BEAN PLANTS

The effect of severe rust infection in reducing the top and seed yield of many plants (2), and, specifically, that of sunflowers (3), is well known. Data on the effect of rust infection on the dry weight of leaves, stems, and new growth of sunflowers in one representative test are given in table 3. In this test, a member of each paired sample was inoculated when the plants had 1 pair of well-developed true leaves and the growth distal to this pair of primary leaves was removed. The growth that occurred distal to the primary leaves after inoculation is considered the new growth. Eleven days after inoculation the weight of the primary leaves of inoculated plants was greater by 15 per cent, the weight of the stems of inoculated plants was less by 12 per cent and the weight of the entire inoculated plants was less by 3 per cent than the weights of paired control plants. By 28 days after

TABLE 3.—*Effect of rust infection on the dry weight of sunflower plants with varying periods of infection*

Plant part	Condition of plants	Mean dry weight in mg. per plant at varying times after inoculation			Difference in percentage[a] mean dry weight of rusted plants compared with controls at varying times after inoculation		
		11 days	20 days	28 days	11 days	20 days	28 days
Total plant, exclusive of roots	Control	128	302	411			
	Rusted	124	256	240	− 3.4 ± 8.8	− 13.9 ± 6.8	− 41.1 ± 6.1
Primary leaves only	Control	44	73	78			
	Rusted	51	83	80	+ 15.2 ± 7.3	+ 13.5 ± 9.7	+ 2.0 ± 4.1
Stem only	Control	84	199	259			
	Rusted	74	148	138	− 12.4 ± 10.2	− 26.4 ± 9.9	− 46.9 ± 4.6
New growth only	Control	0	30	84			
	Rusted	0[b]	7.7	3.0	[b]	− 77.8 ± 42.7	− 96.4 ± 17.9

[a] Mean and standard error of mean for 10 paired samples.
[b] The new growth here was so small that no measurements were made.

inoculation the weight of the primary leaves of inoculated plants was greater by 2 per cent, the weight of the stems of inoculated plants was less by 47 per cent, the weight of the new growth of inoculated plants was less by 96 per cent, and the weight of the entire inoculated plants was less by 41 per cent than the weights of paired control plants. The results at 20 days after inoculation were intermediate. Confirmatory results were secured in 4 other tests, in 2 of which the growth distal to the inoculated primary leaves was not removed at inoculation. In these 2 tests the reduction in the weight of new growth (growth distal to inoculated leaves) due to rust infection was only 12 and 15 per cent at 14 and 15 days after inoculation, respectively, much less than when the new growth was removed at inoculation.

In one test on January 26, 1938, in which the primary leaves of paired young greenhouse Pinto bean plants were inoculated and the growing points were not removed, the dry weight of the entire rusted plants was 15.3 per cent less, and the weight of the infected primary leaves 15.8 per cent greater than the healthy plants 10 days after inoculation. Twenty-one days after inoculation the dry weight of entire plants was 29.6 per cent less and the dry weight of the primary leaves 47.9 per cent greater than the healthy plants. On the basis of this one test the response of bean plants to rust appears to be similar to that of sunflowers.

THE EFFECT OF RUST INFECTION ON THE DIURNAL CHANGES IN DRY-MATTER CONTENT OF BEAN AND SUNFLOWER LEAVES

In tests begun January 3, 1938, 18 days after Pinto beans with one pair of primary leaves had been inoculated with rust, 3-mm. discs were cut from infected and noninfected portions of the same leaves at contrasting times of the day, and weighed (Table 4). To secure closely comparable samples, the afternoon samples were taken from the opposite halves of the same leaves used for the morning samples. In one series (January 3) the afternoon samples were taken first, and in the other 2 series the morning samples were taken first. The rust pustules were large, mostly single, and about 80 per cent of each disc recorded as rusted consisted of infected tissue. The afternoon samples were taken at about 3 p.m. with the hope that they would represent the high phase of the diurnal change in dry weight of leaves (6). The morning samples were taken at 8 a.m. after the plants had been held overnight in a dark chamber.

In these 3 tests, the local increase in dry weight as a result of rust infection varied from 50 to 90 per cent and was always greater in the samples taken at 8 a.m. The diurnal fluctuation was much greater in the noninfected tissue, where it ranged from 15 to 29 per cent of the 8 a.m. value, than in the rusted tissue, where it was about 6 per cent of the 8 a.m. value. Although the differences in dry weight due to time of sampling are not statistically significant for the rusted samples as calculated, the uniformity of the 3 independent determinations must be considered as adding to the significance of the differences.

TABLE 4.—*The effect of rust infection on the diurnal change in the dry matter of bean leaves, 1938*

Series No. and date	Condition of samples	Mean dry weight of leaf samples		Increase of rusted over healthy[a]		Increase of 3 p.m. samples over 8 a.m. samples[b]
		8 a.m.	3 p.m.	8 a.m.	3 p.m.	
		mg. per dcm.²	*mg. per dcm.²*	*Per cent*	*Per cent*	*Per cent*
1. January 3	Healthy	214	277			29.5 ± 6.1
	Rusted	393	416	83.7 ± 5.8	50.2 ± 5.0	5.9 ± 5.1
2. January 5	Healthy	181	220			21.5 ± 6.7
	Rusted	345	367	90.6 ± 9.2	66.8 ± 12.4	6.4 ± 7.6
3. January 6	Healthy	229	263			14.9 ± 5.6
	Rusted	417	442	82.2 ± 6.9	68.0 ± 10.5	6.0 ± 7.2

[a] Mean and standard error of mean for 10 pairs of samples.

[b] Difference and standard error of difference. In this case the standard error is of the difference of the means of independent groups, as there was no pairing of the separate values; for this reason the error is high.

The effect of sunflower rust on the diurnal fluctuation in the dry-matter content of greenhouse sunflower leaves was determined in one test on December 1, 1934, 8 days after inoculation. An estimated 15 per cent of the area of the rusted samples was infected, and the control samples were free of rust. Six paired samples were collected for each time of sampling. The dry weight per square decimeter of leaf was 271 and 290 mg., respectively, for the healthy and rusted paired samples at 5 a.m. and 287 and 297 mg., respectively, at 2 p.m. The increase in dry weight due to rust infection was therefore 3.45 per cent at 2 p.m. and 6.78 per cent at 5 a.m. and the diurnal increase in dry matter (2 p.m. samples as compared to 5 a.m.) was 5.42 per cent for healthy areas and 2.38 per cent for rusted areas.

DISCUSSION

The principal contribution of the present study appears to be the information on the effect of rust infection in increasing the dry weight of infected leaf tissues. The increases in dry weight of leaves due to rust infection varied from -3.1 per cent to $+110$ per cent (Table 1), and most of the low values are apparently due to the low infection in the material studied. From the data of tables 1 and 4 the mean increase in dry weight of leaves due to rust infection was 45.2 ± 8.5 per cent for the 17 paired collections from 11 hosts. If, however, allowance is made for the low percentage area of the samples visibly infected, by assuming the increase in dry weight to be proportional to the percentage area of the samples visibly infected the average increase in dry weight was 61.2 ± 9.9 per cent. The fact that 7 of these 17 samples were bean rust tends to increase the mean.

There is great variation in the effect of a given rust on the dry weight of its host's leaves. This is well illustrated by sunflower rust, with which the measured increase varied from 2 per cent to 46 per cent (Table 2), and by bean rust, in which the measured increase varied from 19 per cent (Table 1) to 91 per cent (Table 4). Three causes of this variation have already been mentioned. The increase is greater with increased severity of infection (Table 1), is greater with increased infection period (Table 2), and is greater for samples taken in the morning than for those taken in the afternoon (Table 4). Several other factors such as environmental conditions would probably also affect the results.

The causes of the increase in dry weight of leaf tissues due to rust infection have not been determined in this study, but an explanation may be suggested by the results obtained. From the data of table 3 and from other data in the text, it is apparent that the increase in dry weight of infected leaves of sunflowers and beans is associated with a decrease in the dry weight of the noninfected stems and new growth, and that the decrease in the weight of stems and new growth is greater than the increase in the weight of the infected leaves. In young plants with 1 pair of primary leaves the new growth and the increase in the weight of the stems with time is due principally to the organic materials photosynthesized in the primary leaves

and translocated to these other parts. Rust infection, therefore, has apparently decreased the translocation of food from the infected leaves. The reasons for this decrease in translocation are probably threefold. First, rust infection probably interferes with the mechanism of photosynthesis so that less total food is formed. The writer has no satisfactory data on this point, but the data of table 4 relative to diurnal variation in dry matter content of bean leaves would tend to support it. Secondly, rust infection greatly increases the respiration of infected tissues (7). This would dissipate *in situ* a considerable portion of the carbohydrates photosynthesized in the leaves. Thirdly, rust infection causes a fixation of considerable dry weight in the infected leaves. It would, therefore, appear that the rust fungi may be nourished principally by the labile organic materials photosynthesized in the leaves, and translocated from them in the case of healthy leaves. That rust fungi cannot be well nourished by the more stable organic materials of leaf tissues is indicated by the paucity of rust development on plants in the dark, unless these plants are supplied with readily available carbohydrates. A somewhat similar line of reasoning has been developed by Mains (5).

SUMMARY

In 10 out of 11 collections of different rusts (on beet, prune, bean, *Cyperus, Polygonum, Xanthium, Iris,* snapdragon, rose, and sunflower) the local dry weight per unit area was greater in rusted than in healthy leaf areas. The differences ranged from −3.0 to +110.0 per cent, with an average of +33 per cent. The rust on poplar proved to be an exception, as measured, but the results may be atypical.

In 14 tests with paired sunflower leaves or plants, the dry weight of the entire infected leaves was from 2 to 46 per cent greater than the dry weight of the paired controls. In 3 tests the green-weight of infected leaves averaged 9 per cent more than that of noninfected leaves and the percentage of dry matter of infected leaves averaged 15 per cent more than that in noninfected leaves.

The reduction in dry-weight yield of entire sunflower and bean plants as a result of rust infection of the primary leaves was associated with a higher yield of infected leaves than of healthy leaves, a lower yield of stems from infected plants than those from healthy plants, and a lower yield of the new growth of infected plants than of healthy plants.

In 3 tests with beans and in 1 test with sunflowers, rust infection decreased the diurnal fluctuation in the dry weight of the leaves.

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SPECIES OF EUTYPELLA AND SCHIZOXYLON ASSOCIATED WITH CANKERS OF MAPLE

ROSS W. DAVIDSON AND ROLLAND C. LORENZ
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INTRODUCTION

During a forest disease survey[1] in the Lake States in the fall of 1935, a study of fungi associated with cankers on sugar maple, *Acer saccharum* Marshall, and red maple, *A. rubrum* Linn., led to the recognition of two distinct cankers, herein referred to as Eutypella[2] and Schizoxylon. As these cankers seemed to be undescribed, preliminary work was done to determine their prevalence and distribution and to establish the pathogenicity of the associated fungi. This work was necessary to ascertain the importance of these cankers in the forest stand and also to determine whether control measures were advisable. The purpose of this paper is to record the characteristics of the cankers, to present data concerning their occurrence, and to describe the fungi associated with them and believed to be their cause.

EUTYPELLA CANKER

Distribution and Prevalence

A canker, with which a species of *Eutypella* was consistently associated, was found on sugar and red maples throughout all the national forests of the Lake States. It was most abundant in the Chequamegon, Nicolet, Ottawa, and Upper Michigan National Forests. The fact that it also has been collected by one of the writers and others[3] on cankered sugar and red maples outside of the Lake States indicates that it occurs over an extensive area.

[1] Lorenz, R. C. and Christensen, Clyde M. A survey of forest tree diseases and their relation to stand improvement in the Lake and Central States. October 7, 1937. (Mimeographed.)

[2] In 1933 Frank Kaufert, Pathologist, Pest Control Research Section, Du Pont Laboratories, observed and collected this type of canker on sugar maple in Itasca State Park, Minnesota, but no determination of the associated fungus was made. (Unpublished report.)

[3] Specimens of this canker and the associated *Eutypella* on sugar maple were collected by other workers in the following localities and sent to the writers for identification: Carrol, New Hampshire, May 12, 1933, by J. R. Hansbrough and T. J. Grant; West Burke, Vermont, Oct. 11, 1933, by D. S. Welch (No. 1343); Seventh Lake, New York, Aug. 25, 1934, by D. S. Welch and F. C. Stewart (No. 1342); Carrol, New Hampshire, May, 1935, by T. J. Grant; Gale River, New Hampshire, Nov., 1937, and Danby, Vermont, May, 1938, by W. A. Campbell.

Cankers believed to be caused by this species of *Eutypella* were found much less frequently on red than on sugar maple. Sample plots 1/10 acre in area indicated that 7 per cent of the sugar maples were affected on the Chequamegon National Forest and that most of the diseased trees were of the suppressed and intermediate crown classes. In the mature stand of northern hardwoods near the James Lake Camp on the Ottawa National Forest, about 2 sugar-maple trees per acre were cankered. The canker was common on sugar maple in the vicinity of the Argonne ranger station on the Nicolet National Forest. On the Dukes Experimental Forest, in upper Michigan, the disease was common on sugar maple of all age and crown classes and appeared to be more prevalent there on older trees than elsewhere. Only an occasional cankered tree was found in the Cadillac ranger district of the Manistee National Forest, where northern hardwoods are common. From 20 to 30 cankered trees per acre occurred in a pure stand of young sugar maple near Cloquet, Minnesota.

Economic Importance

Trees over 5 inches diameter breast height are seldom killed by this disease, but smaller trees, especially those suppressed in the 1- to 3-inch diameter classes, often are killed. The death of some of these trees is probably due to the combined effect of the fungus and suppression, but small trees not suppressed may be killed. Cankers persist on the larger trees for many years and occasionally reach a length of 5 feet (Fig. 2, C). Such trees are liable to breakage at the canker.

At present it seems likely that this disease is of importance only in those areas that contain a high proportion of sugar maple. It does not appear to be doing much damage in the well-mixed stands of northern hardwoods, which are comprised chiefly of birch, beech, maple, and hemlock.

Description of the Canker

Eutypella cankers are characterized in general by firmly attached bark, heavy white to buff mycelial fans under the bark at margins, broad, slightly raised concentric rings of callus tissue, and the presence of long-beak perithecia with their black, sulcate ostioles protruding above the bark in the centers of old cankers. They are usually similar in appearance to Strumella cankers.

The bark becomes tightly attached to the wood by the dense mycelial mat. This mycelial layer (Fig. 1, A) is at first white to buff, consisting of radiating strands of hyphae closely matted together, but it gradually darkens and coalesces with the bark and wood. The heavy mycelial fan development, which is always present, is the most characteristic feature of this canker.

The annual extension of cankered areas is usually uniform, averaging about $\frac{1}{2}$ inch (Fig. 1, A). However, this extension is occasionally checked suddenly by some unknown cause, around either the entire margin or on only a portion of it. Such sudden cessation of the extension gives the canker a

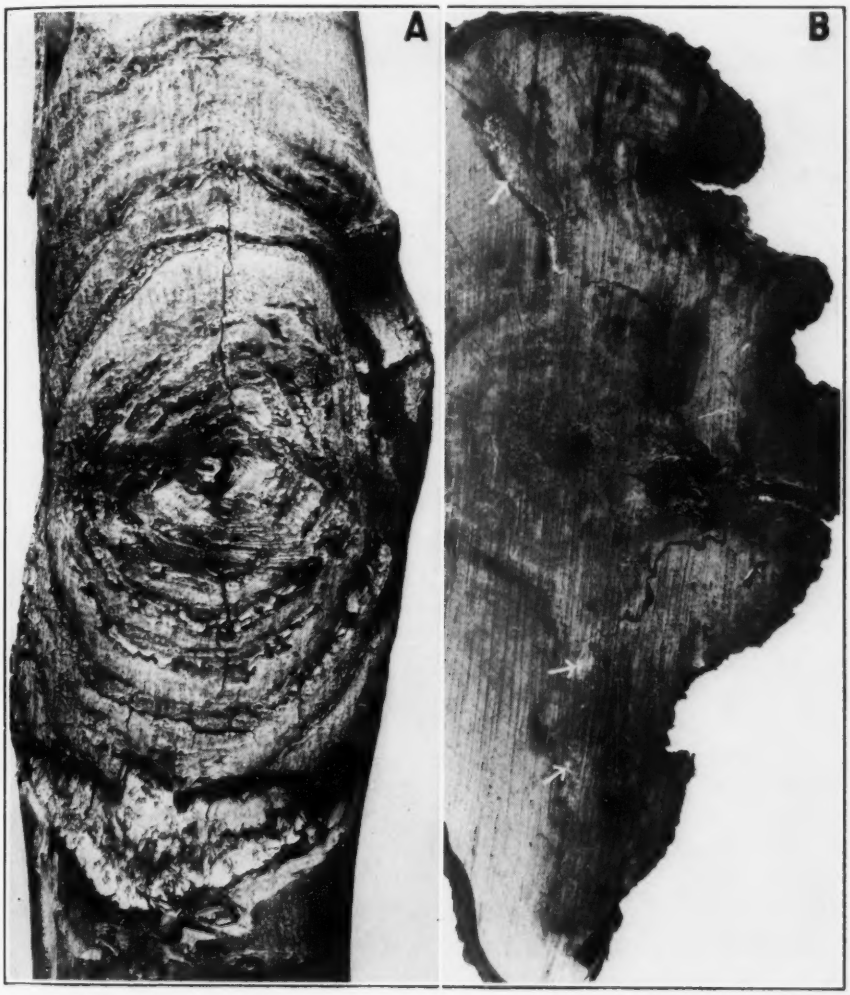

FIG. 1. A. Young Eutypella canker on 3.5-inch trunk of red maple. Perithecia are abundant in center of this canker. Note mycelial fans at lower margin of canker where bark has been removed. B. Cross section of old canker on 10-inch sugar maple. Arrows show white mycelium of *Eutypella* growing out along margin of diseased and healthy wood. Decay in center of this section was caused by *Polyporus glomeratus*. Photographs by M. L. F. Foubert.

rough or irregular appearance, whereas, normally, it would be relatively uniform, and as a tree bearing such a canker becomes older, considerable distortion of the stem results (Fig. 2, A to C).

Eutypella penetrates deeply into the sapwood beneath the canker and usually extends beyond the center of the tree. It also grows slowly upward and downward, forming a central column of discolored wood. Infected wood near the center of the canker finally becomes brown and brittle in appearance and seems to be slightly decayed. A single species of *Eutypella*

has been consistently isolated from this discolored wood—both from under the center of cankers and from near the margins of the discoloration. This ability of the fungus to penetrate the wood probably prevents the cankers from becoming permanently inactive.

Older cankers usually contain perithecia imbedded in the outer bark of the central portion and their necks protrude slightly above the surface, giving the bark a blackened appearance. Perithecia have not been observed to develop nearer than the fourth or fifth annual callus ring from the margin of cankers, and frequently they are confined to only a small central portion

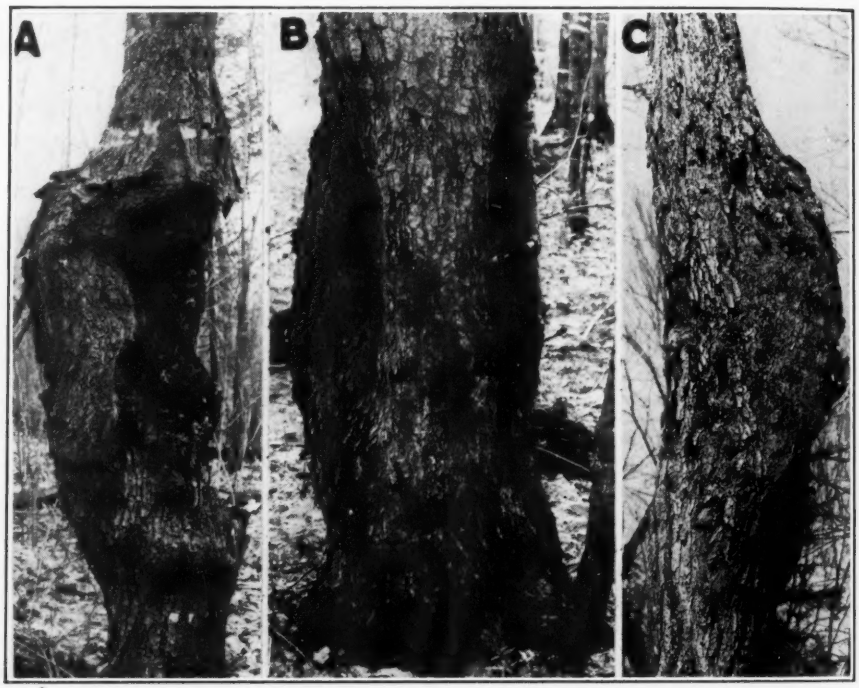

FIG. 2. Eutypella cankers on sugar maple showing manner in which the trunks are deformed. A. Cankered 6-inch stem. B. Large canker at base of trunk. C. Canker 5 feet in length and about 10 feet up from base of 12-inch stem.

of large cankers. It seems probable that fruiting depends on thorough invasion of the wood, which is apt to occur sooner under the central portion. Cankers collected at various times indicate that mature perithecia persist throughout the year. That is, whenever perithecia were present mature viable spores could be obtained from most of them.

Initial infection apparently occurs when the tree is young, since no young infections ever were found on older trees; but, once a tree is infected, development continues for many years. Cankers usually occur at heights of 2 to 8 feet aboveground, but have been observed in a few cases at ground line or at a height of 30 feet. All observed cankers have been on the main stems, but dead branch stubs were usually present at their centers. Usually, there is only one canker on a tree.

Inoculations

In the summer of 1936, Lorenz and Kaufert inoculated numerous sugar-maple trees with *Eutypella*. In the spring of 1937, 3 of these trees were cut and the fungus reisolated from the host tissue surrounding the point of inoculation in each case. A small area of cambium around the inoculation injuries was dead, but insufficient time had elapsed for typical canker formation to be expected. Check injuries on these same trees gave no *Eutypella* in culture when isolation attempts were made. The information is, however, considered insufficient for proof of parasitism.

Associated Fungus

Isolation Results: There were no fruiting bodies on the first specimen of this disease, which was sent to the Division of Forest Pathology in the fall of 1935; but numerous isolates from both the cambium at the margin of the canker and the discolored wood beneath uniformly consisted of a white mycelium, which, in 4 to 6 weeks, bore an abundance of *Libertella* spores. Many older cankers collected later contained mature perithecia of a species of *Eutypella*, the ascospore cultures of which were similar in every respect to the cultures isolated from the host tissue.

Isolations were later made from the margins of a number of cankers of this type and from discolored wood beneath the cankers and cultures of the above-mentioned fungus were consistently obtained. Even from the brittle brown wood frequently present under the center of the canker, the fungus was usually isolated in pure culture. This would indicate that when this species of *Eutypella* is well-established in the wood, other fungi usually are excluded. This characteristic also is shown by the fact that, even where insects have tunnelled through the wood, pure cultures of it usually are obtained. Figure 1, B shows a cross section of a large canker on an old sugar maple that contained decay (*Polyporus glomeratus* Pk.) in the central portion. It will be seen that a black line had formed around the decay on the side next to the canker. From inside this black line, only the decay fungus was obtained in culture, while from the brown, brittle wood on the outside of the black line, only *Eutypella* was isolated. The same cross section shows white mycelium of *Eutypella* growing out along the line between the brown discoloration and the living sapwood.

Cultural Characteristics of the Eutypella: The *Eutypella* grows readily on various culture media such as malt, potato dextrose, and corn meal, but a 2.5 per cent malt (Trommers) agar medium was used throughout this study. The mycelium is fairly dense and remains white (Fig. 4, A and B), except in older test-tube cultures, where it may develop patches of light buff to cream color. The color is usually present at the lower margin of the slanted agar, where the mycelium grows between the glass side of the tube and the agar. It is in such positions that spores usually are produced. The long, curved spores (Fig. 5, H) are not formed in a pycnidium but occur in great abundance on an indistinct layer in the mycelium. The cultures usually require

a 4- to 6-week incubation period at room temperature (25° C.) for spore production. Perithecia have not been obtained in culture.

As there are a number of closely related genera having conidia similar to those of the form genus *Libertella*, any attempt at identification from cultural characteristics would have to take into consideration species of *Diatrype*, *Diatrypella*, *Eutypa*, *Valsella*, and probably several others, as well as those of *Eutypella*. Cultures of only one other species of *Eutypella* have been available for such comparison, but a number of ascospore cultures of species from related genera have been used, and the preliminary study indicates that identification on the basis of cultural differences might be possible. *Eutypella angulosa* (Nits.) Sacc. from yellow birch, *Betula lutea* Michaux, for instance, grows more rapidly in Petri-dish cultures; and conidia, red in mass, are produced at the top of test-tube slants. Conidia of *E. angulosa* (Fig. 5, I) are also shorter and less curved than those of *Eutypella* from cankered maple. Ascospore cultures of *Eutypa heteracantha* Sacc.[4] from *Ailanthus* develop an abundance of dark mycelium and its conidia (Fig. 5, J) differ slightly from those of the canker fungus. *Diatrype hochelagae* E. & E., which was collected several times on old cankered areas of maple stems during the present studies, grows much more slowly in culture and has shorter less curved conidia, somewhat similar to those of *Eutypella angulosa* as shown in figure 5, I. Numerous other common species of *Diatrype* such as *D. stigma* (Hoffm.) Fr. and *D. virescens* (Schw.) Cke., and *Diatrypella prominens* Howe, which have been grown in culture but not studied critically, are not likely to be confused in culture with this particular maple fungus, although they have somewhat similar conidial stages. Cultural comparisons should eventually be made between the common saprophytic forms reported on maples and the canker form.

Comparison with Other Species of Eutypella on Maple: Although no species of *Eutypella* or *Eutypa* is known to have been reported in the literature as causing or occurring on cankers on maples, several of the described saprophytic species are somewhat similar to the one we have found occurring on cankers. *Eutypella corynostoma* (B. and Rav.) Sacc. and *E. constellata* (B. and C.) E. and E., which occur on maple, have ascospores of about the same size. Also, the general structure and arrangement of perithecia are similar to those on the cankers.

The ascospores from the cankered specimens seem to be consistently darker and less curved than those examined from the above-mentioned saprophytic forms. These spore characters are consistent for all specimens examined, regardless of apparent differences in age of stromata. Another respect in which the canker strain differs is in its heavier stromatic development, which might result from its restricted area of growth throughout its long period of activity. Because of these slight differences and its association with a definite canker, we are not assigning it to any previously named species. Since no extensive monographic study has been published on *Eutypella* and

[4] This and several of the other species referred to were identified by C. L. Shear, formerly principal pathologist, United States Department of Agriculture.

related genera, it has not been possible to make satisfactory comparisons with the many inadequately described species. For convenience of future workers this *Eutypella*, which, so far as is at present known, occurs only on cankers on maple, is named and described as a new species. A type specimen has been deposited in the mycological collections of the Bureau of Plant Industry, Washington, D. C.

Technical Description: **Eutypella** (*Eutypa*) **parasitica,** n. sp. (Fig. 5, F to H).

Pustules usually densely grouped, 3 to 15 mm. in diameter, or coalescent over large areas with closely aggregated ostioles blackening the fissures in the bark; perithecia irregularly crowded together in the slightly altered outer bark (usually in bark closely attached to the wood near center of canker) in groups of 10–40 (sometimes in much greater number), and sometimes imbedded in wood of the central branch stub, occasionally circumscribed by inconspicuous black line, black, large 0.6 to 0.9 mm. diameter; necks long, extending slightly beyond surface of the bark or, when in deep fissures, long and protruding toward the light, cylindric, sulcate at the ostiole; asci small, stipitate, spore-bearing part 32–40 × 6–7 μ, stipe 10–40 × 1.5 μ; ascospores very slightly curved, irregularly 2- or-more seriate, dark (smoky) brown, slightly attenuated at ends, 8–11 × 2–2.3 μ; conidia (found in culture only) long, slender, pointed at the ends, 26–34 × 1 μ, curved (mostly U-shape), hyaline, only slightly buff-color or hyaline in mass.

Growth rate[5] in culture, about 20 mm. in 7 days. Constant temperature studies show good growth at 20° to 35° C., no growth or only a trace at 40° C.

Pustulis orbicularibus gregariis, 3–15 mm. diam. interdum majoribus, in corticali immerso, ostiolis erumpentibus, peritheciis inordinatis congestis, interdum in ligno immersis, 10–40 vel pluribus in stromate singulo, nigris, subglobosis, magnis, 0.6–0.9 mm. diam.; ostiolis longissime exsertis, cylindraceis, sulcatis; ascis cylindrico-clavatis, filiformi-stipitatis, octosporis, p. sp. 32–40 × 6–7 μ, stipite 10–40 × 1.5 μ; ascosporis subdistichis, leniter curvatis, fuscis, 8–11 × 2–2.3 μ; conidiis (in culturis productis) cylindricis, curvulis, utrinque attenuatis, hyalinis, 26–34 × 1 μ.

On cankered maples, *Acer saccharum* Marshall and *A. rubrum* Linn. in Minnesota, Wisconsin, Michigan, New York, New Hampshire, and Vermont. Herbarium specimens 71988 type, 71656, 71001, 71011, 71624, 71110, and 70999.

SCHIZOXYLON CANKER

Distribution, Importance, and Prevalence

Another type of canker, the Schizoxylon canker, was found occasionally on both red and sugar maples in the Chequamegon, Ottawa, Upper Michigan, and Manistee National Forests, but it has not been found outside the Lake States. The information so far obtained indicates that the Schizoxylon canker is of little importance, because it is rare and observed cankers were found usually on suppressed trees less than 3 inches in diameter.

[5] Average radial growth at room temperature of about 25° C.

Description of the Canker

Schizoxylon cankers resemble Nectria cankers. Their annual extension, as indicated by callus folds, is much less than that of those associated with *Eutypella* and results in a smaller, more sunken canker. As the bark remains attached ordinarily for only a few years, exposed wood is present in the center (Fig. 3, A and B). This exposed wood is weathered and often

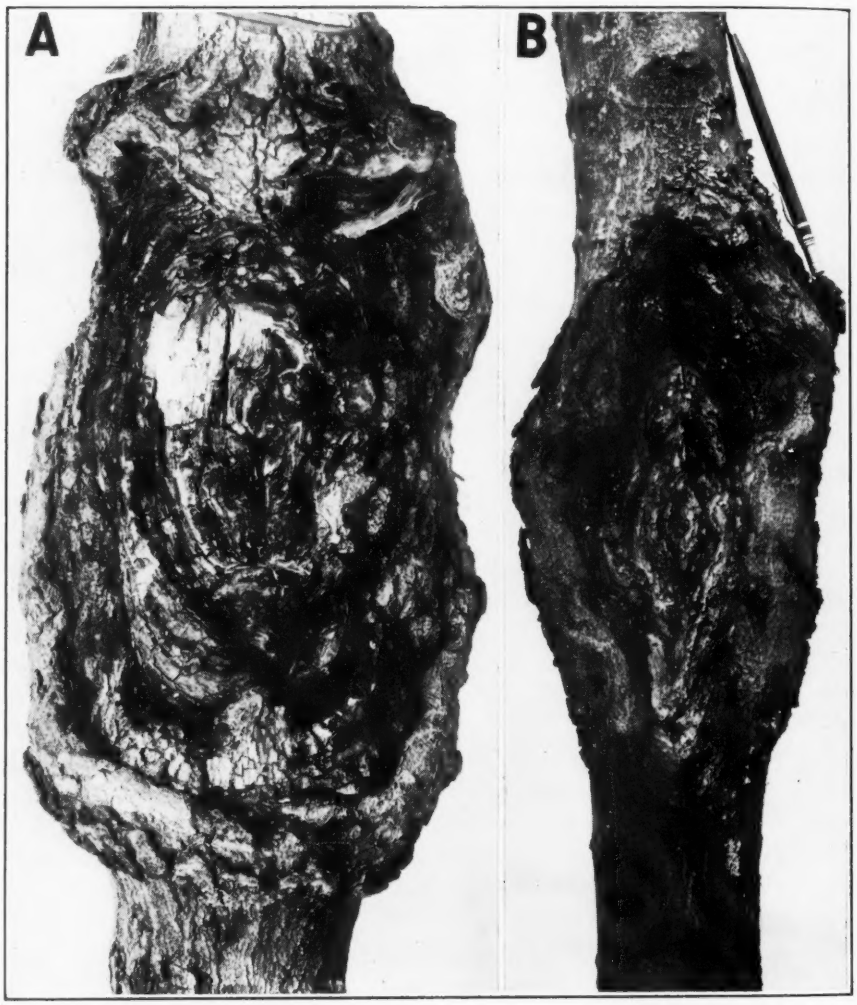

FIG. 3. Schizoxylon cankers. A. Old canker on sugar maple. B. Similar canker on red maple.

partly decayed on the surface. The interior of the infected trunks is discolored, as described for Eutypella-infected trees.

The margins of the cankers where the fungus is active are characterized by the presence of white mycelial fans, which are less conspicuous than those on Eutypella cankers.

The cankers are often irregular, and activity may be entirely checked for a time or may continue in only a small portion of the margin. This activity usually does not cease until the diameter of the cankers is equal to or greater than the diameter of the trunk. It is accompanied by a distortion of the trunks on which it occurs. This is at first manifested by a flattening and later by a protruding margin of thickened callus tissue (Fig. 3, A and B).

The light-grey pycnidia of the fungus can readily be seen sunken in the exposed wood and are often present but less conspicuous in the bark near the margin of the canker.

Isolation Results

Schizoxylon was isolated from the margins of a number of cankers, but the percentage of successful isolations was lower than for the *Eutypella*. It was also frequently isolated from the discolored wood near the surface and occasionally from the internal discoloration, but miscellaneous unidentified fungi were also frequently obtained from the discolored areas. The extremely slow growth of *Schizoxylon* in culture may account for this low percentage of successful isolations in that other wood-inhabiting fungi would suppress it.

Cultures were readily obtained from the pycnospores and were identical with those from wood and bark.

Inoculations

A number of inoculations were made with Schizoxylon at the time when the *Eutypella* inoculations were made. Of three inoculations removed in 1937 reisolations were successful in only one case. In the case of the successful reisolation pycnidia of *Schizoxylon* were abundant on the bark around the point of inoculation. The white mycelial growth had penetrated the bark surrounding the injury, but the cambium was not killed back to any marked extent. The other two inoculations showed no indication of the fungus' having become established and molds and other fungi had grown over the injured area. *Schizoxylon* was not isolated from check injuries on these same trees.

Description of Schizoxylon

In Culture: Schizoxylon grew on various culture media, but 2.5 per cent male agar was used for isolations and growth-rate studies and Difco cornmeal agar for spore-germination work.

This fungus has a characteristic white mycelial mat (Fig. 4, C and D), which, coupled with the slow growth rate, permits easy identification. In fact, it could not be confused with any fungus isolated in the course of these studies. The mycelium is white, but occasional light brown patches occur in older cultures. The mat has a tufted, wavy appearance with numerous globose mycelial bunches that appear to be pycnidial primordia. The margins are thin and irregular.

Fruiting in Nature: When maple cankers were first collected for a study of possible causal organisms it was noticed that a few that resembled

Nectria cankers in general appearance contained light gray pycnidia imbedded in shallow cup-like depressions in the bark and wood. These pycnidia were globose and had no ostioles, but their interiors were filled with a compact mass of small rod-shape spores.

The appearance of the pycnidia suggested that a discomycete, similar to *Stictis*, might be the perfect stage. A search of the literature on *Stictis* and

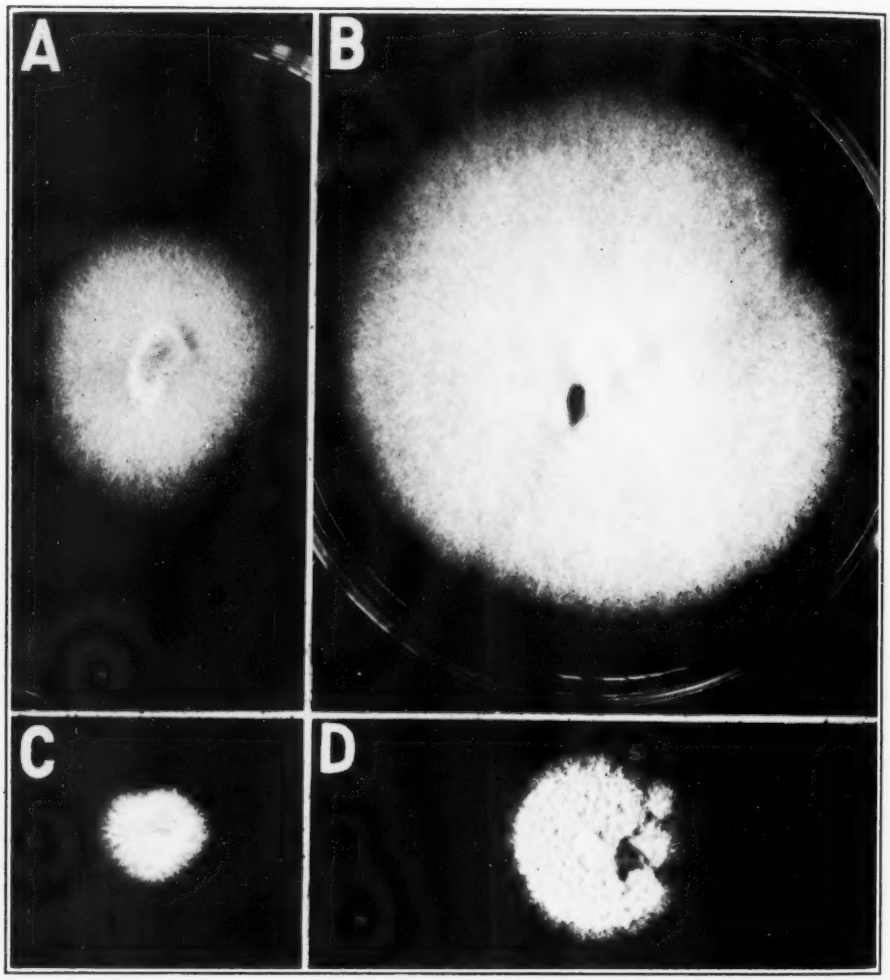

FIG. 4. A and B. 7- and 14-day-old cultures of *Eutypella parasitica*. C and D. 7- and 14-day-old cultures of *Schizoxylon microsporum*.

related genera disclosed only one species, namely *Schizoxylon sepincola* Pers.,[6] reported as having a pycnidial stage. Continued collection of cankers in an attempt to find such a discomycete finally resulted in obtaining one on red maple at Baldwin, Michigan, with a species of *Schizoxylon* fruiting on it. This same canker had typical pycnidia fruiting in association with the

[6] Tulasne. Selecta Fungorum Carpologia. V. 3, page 148. 1865.

apothecia. In appearance the apothecia of this *Schizoxylon* were similar to the pycnidia, except for a small dark opening on top (Fig. 5, K). The interior was dark and hollow in the upper part with a compact layer of asci below. This canker form has no branched paraphyses as are described for *S. sepincola* and its ascospores are smaller. The pycnidial stage is apparently quite similar to that recorded for *S. sepincola*.

Numerous cultures obtained from the small segments of ascospores and from single asci containing germinating spores were similar to the cultures from pycnospores and host tissue.

The *Schizoxylon* found fruiting on the one red-maple canker does not fit the descriptions of any described species.[7] It more nearly fits descriptions of *S. sepincola*, but does not have branched paraphyses and its ascospores are narrower. It is, therefore, described as a new species.

A type specimen has been deposited in the mycological collections of the Bureau of Plant Industry, Washington, D. C.

Technical Description: **Schizoxylon microsporum,** n. sp. (Fig. 4, C and D and Fig. 5, A to E and K).

Apothecia rare, scattered singly or clustered, sunken in the bark or exposed wood, 0.7 to 1 mm. diam., at first covered by a white papillate membrane (black around ostiole) which splits back from the central opening finally flask or cup-shape; asci in a thick layer filling the lower half of the globose fruiting body, 170–200 × 8–12 μ, wall thickened at tip, cylindric, tapering slightly to short pedicel; paraphyses filiform, as long as the asci, septate, not (or only slightly) thickened above, not branched, 1.3 to 1.7 μ diam.; ascospores filiform, almost as long as the asci, multiseptate, 1 μ diam. or less, finally breaking up into short rods or slightly curved segments 2–3 × 1 μ, sometimes longer and septate (4–8 × 1 μ).

Pycnidia common, imbedded in bark or surface of wood, globose 0.6 to 1 mm., white to light gray, similar to apothecia but without papillate opening; spores histogenic, filling pycnidium in compact hyaline mass, small, often slightly curved, 2–5 × 1 μ. Growth rate 5 to 6 mm. in 7 days at ordinary room temperature of about 25° C., growing well at temperatures of 15° to 30° C.

Ascomatibus raris, in cortice et ligno initio subimmersis, dein erumpentibus, singulis vel gregariis, disco membrana pulveracea griseoalba tecto, ostiolo punctiforme, 0.7–1.0 mm. diam.; ascis cylindraceis, brevipedicellatis, 170–200 × 8–12 μ, crasse tunicatis; paraphysibus filiformibus, simplicibus, pluriseptatis 1.3–1.7 μ diam; sporidiis filiformibus, parallelis ascorum longitudine, 1 μ diam. pluriseptatis, mox in articulos 2–3 μ longos secedentibus.

Pycnidis communibus, in cortice et ligno subimmersis, erumpentibus, membrana pulveracea griseo-alba tectis, singulis vel gregariis, ostiolo carente; pycnoconidiis histogenicis, pycnidium implentibus, cylindraceis, rectis curvatives, 2–5 × 1 μ.

[7] The authors wish to acknowledge the assistance of Edith K. Cash, Assistant Mycologist, United States Department of Agriculture, in attempts to identify this fungus and in writing the Latin descriptions.

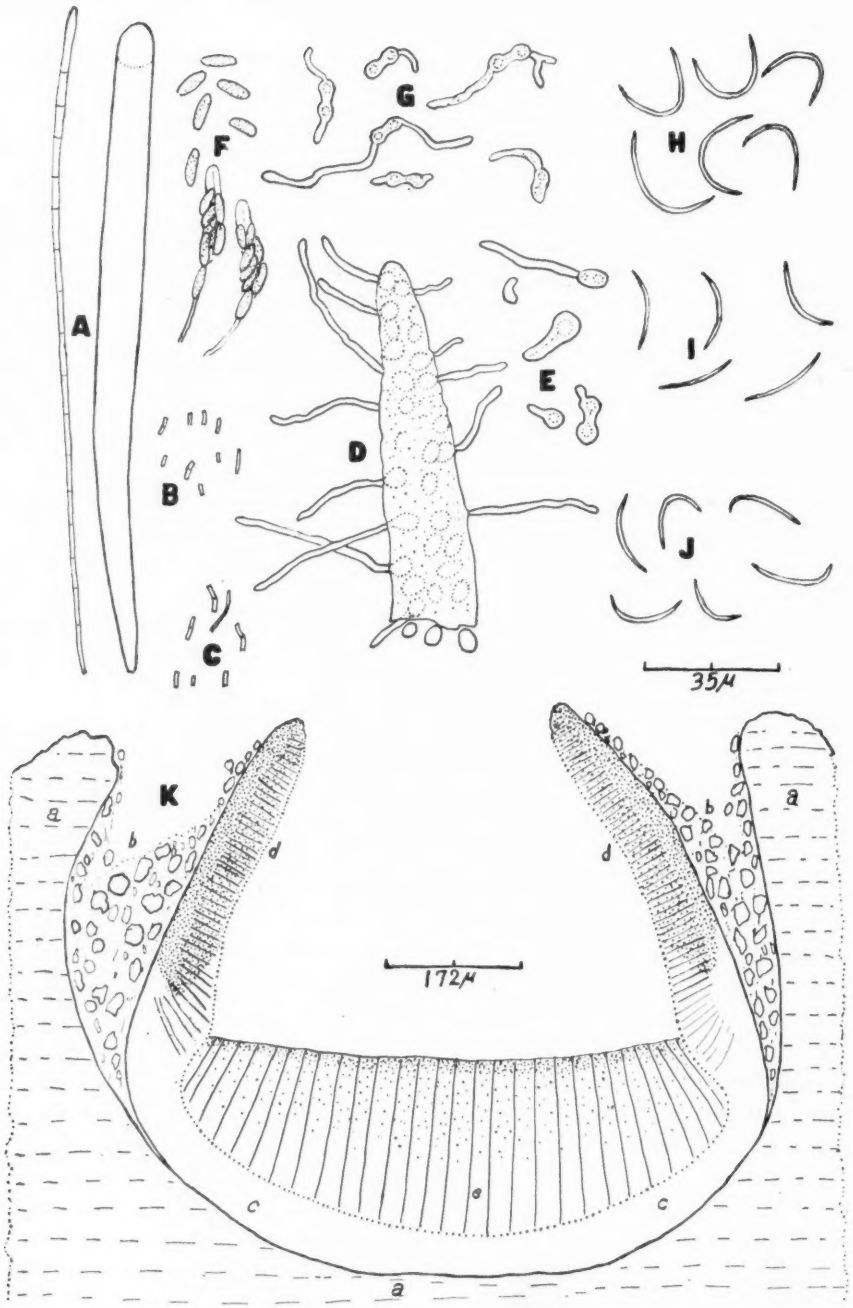

FIG. 5. A to E and K. *Schizoxylon microsporum*, n. sp. A. Paraphysis and ascus. B. Segments of ascospores. C. Pycnospores. D. Tip of ascus after 20 hours on corn-meal agar showing germination of ascospores. E. Segments of ascospores showing early stages of germination. F to H. *Eutypella parasitica*, n. sp. from maple canker. F. Asci and ascospores. H. Conidia from 6-week-old culture. I. Conidia from ascospore culture of *Eutypella angulosa*. J. Conidia from ascospore culture of *Eutypa heteracantha*. K. Sketch of a section of apothecium of *Schizoxylon microsporum:* a, wood; b, white crystalline material; c, hypothecial layer; d, black upper covering; e, ascus layer.

On cankered *Acer saccharum* Marshall and *A. rubrum* Linn. in Minnesota, Wisconsin, and Michigan. Specimen number 71569 type, pycnidial specimen Nos. 71450, 71451, and 71571.

SUMMARY

Two cankers, *Eutypella* and *Schizoxylon*, are reported on sugar and red maples and described for the first time. Eutypella canker, which has dense white to light buff mycelial fans under the bark at the margin, has *Eutypella parasitica*, n. sp. commonly fruiting on it. The other has associated with it the pycnidial stage of *Schizoxylon microsporum*, n. sp. The ascus stage was found on one canker and cultures from ascospores were the same as cultures from pycnospores.

The Eutypella canker is common in the Lake States and has been collected in New York, Vermont, and New Hampshire. Schizoxylon canker occurs only occasionally throughout the Lake States. Both appear to cause conspicuous killing of the bark and distortion of the trunk.

Both fungi have been repeatedly isolated from margins of active cankers and from discolored wood back of killed areas, and such isolates appear to be identical with cultures from ascospores. A few inoculations were made on sugar maple, and the fungi were reisolated in a few cases but insufficient time had elapsed to get typical canker formation.

DIVISION OF FOREST PATHOLOGY, BUREAU OF
PLANT INDUSTRY, AND CIVILIAN CONSERVATION CORPS,
UNITED STATES DEPARTMENT OF AGRICULTURE.

GRAVITY GRADING, A METHOD FOR REDUCING SEED-BORNE DISEASE IN COTTON

K. STARR CHESTER

(Accepted for publication July 16, 1938)

Cottonseed is commonly infected to a serious extent with pathogenic organisms, particularly *Glomerella gossypii* Edg., *Bacterium malvacearum* E. F. S., *Fusarium moniliforme* Shel. and other species of *Fusarium*. These organisms are present in the lint and on the surface of the seed, as well as within the seed coat. They are so destructive in the stages of germination and emergence that it is not uncommon to use a seeding rate 10 or more times in excess of the expected stand. Of the various suggested methods for controlling cottonseed pathogens, 2 are outstanding in their beneficial effects, *i.e.*, delinting with concentrated sulphuric acid, and dusting with organic mercury preparations such as 2% Ceresan. While these 2 means of surface disinfection are advantageous, they do not completely solve the problem of cottonseed treatment, since neither will prevent the ultimate activity of pathogens within the seed coat. The planting of a single internally-infected seed in a hill is a hazard to the remaining healthy seed in the same hill.

Even acid- or Ceresan-treated seed lots commonly contain 10 per cent or more of internally infected seed, and these are capable of infecting many of the remaining healthy seed before the stand is established. The purpose of this paper is to report a practical method that to a large extent eliminates the internally infected seed.

Briefly, the method consists of acid-delinting followed by covering the seed with water and rejecting the fraction that floats. The acid-delinting is carried out according to standard methods using 9 lb. ($\frac{1}{2}$ gal.) of 66° Baumé sulphuric acid per bushel of fuzzy seed. Half of this amount of acid is sufficient if gin-delinted seed are used. The delinted seed are washed in the usual manner. When washing is complete, the seed are allowed to settle in the water, and the fraction which rises to the surface is decanted or removed with a screen. The two fractions, hereinafter referred to as "light" and "heavy," are dried and bagged separately. Ordinarily, about 40 per cent of the whole seed, by weight, fall into the light fraction (average of tests of 19 seed lots). The heavy fraction is used for planting; the light fraction may be sold to cottonseed processors at a satisfactory price.

The superior quality of the heavy fraction has been seen in numerous laboratory, greenhouse, and field tests. The results of these tests are here briefly summarized, and details of the tests are reserved for a later publication.

Laboratory germination tests comprised 180 germinations of 26 lots of cottonseed. The "rag doll" method was followed using 100 seed to a "doll," and only "clean germination" was scored, *i.e.*, seedlings with obvious infection such as to preclude survival were classed with ungerminated seed. Ordinarily, a test consisted of the germinations of 4 samples from the same source: (a) fuzzy seed, (b) the same after delinting but without further fractionation, (c) the light fraction of (b), and (d) the heavy fraction of (b). Averaging all experiments, the heavy fraction (d) proved to be considerably superior to all other samples. The heavy seed produced 2.2 times as many healthy seedlings as the light seed, and 1.24 times as many healthy seedlings as the whole delinted fraction (b). In many cases the heavy seed produced 99 or 100 healthy seedlings from 100 seed, while the whole delinted fraction produced about 80 healthy seedlings and the light fraction about 50.

The percentage of germination, however, gives only a partial measure of the superiority of the heavy seed, since the seedlings from heavy seed proved more vigorous than the seedlings from the other fractions.

Whole delinted seed usually produced a higher percentage of healthy seedlings than fuzzy seed of the same source. However, the more striking gain in clean germination after fractionating the delinted seed indicates that internal infections are more important in cottonseed pathology than the surface infestations, which can be controlled by chemical methods.

Greenhouse germination tests in sterilized and in unsterilized soil gave essentially the same results as the laboratory germination tests.

In the field, the method was tested in 3 plantings in the spring of 1938. In each planting the seed were commercial lots from local sources, four randomized replications were used, and the heavy seed were tested in comparison with light seed, whole delinted seed, and fuzzy seed, all originally from the same sack. Each of the seed lots was hand-planted in four 50 ft. rows in hills of exactly 5 seed each, at 10″ intervals. The weather was well adapted to a test of the method under both favorable and adverse conditions.

Planting No. 1 (April 24) was followed by cool wet weather. The same planting included 16 fuzzy seed lots, some treated with "Ceresan" (Cotton Disease Council, 1938 cooperative seed treatment tests). Of the 80 rows planted, only 4 produced useable stands; these 4 were the 4 replications of heavy delinted seed. The heavy seed produced 4.5 times as many seedlings per row as the average for the 19 remaining seed lots.

Planting No. 2 (May 13) was followed by warm rains. This planting was an exact repetition of planting No. 1 except that the randomization was independent. Most of the seed lots gave stands that were usable, although not outstanding. Averaging the four replications, the heavy seed fraction produced twice as many healthy seedlings as the whole delinted fraction and 3 times as many as the light fraction.

Planting No. 3 (May 21) was followed by ideal conditions for cottonseed germination. The planting was also as in the former two, but independently randomized. The stands of the better seed were excellent. The four replications gave uniform results with relatively small variation. The averages of the replications showed a healthy emergence of the heavy fraction, which was 1.5 times as great as that of the whole delinted seed and 2.4 times as great as that of the light fraction.

The field experiments afford further evidence that the heavy fraction of delinted seed produces a significantly higher percentage of healthy seedlings than whole delinted seed. This superiority of the heavy fraction was maintained under poor, average, and good conditions for germination. The agreement of field, greenhouse, and laboratory tests, and the similarity of the results obtained with numerous seed lots, indicate that the method of gravity-grading appears to have a general application in cottonseed improvement. As yet we are unable to state the effect of gravity-grading on the yields or quality of cotton. However, it is known that the yields from even stands usually exceed those from broken stands, and an increased yield would be anticipated from any practice that tends to eliminate skips in the row.

The use of gravity-grading in farm practice appears to be practicable. Acid delinting has not become an established practice in many parts of the cotton belt, largely because of the lack of suitable equipment. At present the grower is limited to two alternatives, home delinting in tubs, or the installation of the seed-delinting machinery devised in Arizona.[1] The home

[1] BROWN, J. G., and F. GIBSON. A machine for treating cotton seed with sulphuric acid. Ariz. Agr. Exper. Sta. Bull. 105. 1925.

method is unpopular because the average cotton grower is unable to handle concentrated sulphuric acid with safety and efficiency. The Arizona machinery is expensive to construct and operate, and in some cotton areas its cost may be prohibitive. It is believed that delinting machinery can be constructed that will be reasonably inexpensive and that can be made generally available on a community basis, at a low cost. Provided the expense of delinting equipment is not excessive, the cost of delinting and fractionating is more than offset by the following facts:

1. Heavy delinted seed can be sown at $\frac{1}{3}$ the seeding rate for fuzzy seed, and still produce good stands under optimum conditions.
2. The light fraction can be sold for a satisfactory price as is indicated by such quotations from cottonseed processors as the following:

   "On a clean seed basis, our laboratory finds that the total oil is 323 lb. per ton and the available oil 258 lb., and on the basis of 5% manufacturing loss these seed will produce 1,028 lb. of 43 per cent meal to the ton. Such seed would be desirable for crushing purposes, and we believe that any mill would buy them in any quantity on the basis of the market value of prime seed." (Southwestern Cotton Oil Co., Oklahoma City, Okla.)

   The light fraction may also be suggested for trial as a superior livestock feed by virtue of its low percentage of fiber. Most or all of the price received for the light fraction is profit, since the heavy fraction can be used to plant an acreage equal or nearly equal to that that would otherwise be planted with whole seed.
3. The heavy seed are not only relatively disease-free but, in addition, they represent the seed with the largest amounts of reserve food. This implies greater vigor throughout the early period of seedling development.

So far as is known to the writer, the only previous reports on the weight of cottonseed in relation to successful culture are those of Kottur, Staten, and Taubenhaus and Burkett. Kottur in India applied a brime flotation technique to fuzzy seed and reported better germination with the heavy fraction.[2] He stated that the method was not applicable to American seed. We have attempted this method, with additional variations, and in no case have been able to obtain a satisfactory fractionation of American seed. Staten in 1933[3] weighed individual seed and showed that the heavier seed produce more vigorous plants. These gave larger yields than the plants from small seed during the first year after selection. Yields the second year were reported to be less in favor of the heavy seed. No method was proposed for the practical application of this finding. It should be pointed out that our method differs from that of Staten in that it depends

[2] KOTTUR, G. L. A simple method of selecting heavy seeds in cotton. Poona Agr. Col. Mag. **8**: 203–210. 1917. (Abstracted in Experiment Station Record **40**: 237–238. 1919.)

[3] STATEN, H. W. The comparative effects of light and heavy seed in cotton. Proc. Assoc. So. Agr. Workers **34**: 84. 1933.

upon the specific gravity of the seed and not on their absolute weight. Measurements by G. Tennyson[4] indicate that there is little or no correlation between absolute weight and specific gravity of cottonseed after eliminating a small fraction of immature seeds and empty hulls. Internally infected seed are well distributed through all of the absolute weight classes.

In the Texas Agricultural Experiment Station Report for 1936[5] Taubenhaus and Burkett very briefly mention grading delinted cottonseed by the use of specific gravity. Irrespective of subsequent treatments with fungicides, the seed that were heavier than water were superior to ungraded control samples as regards germination percentage, rapidity of emergence, and final stand produced.

The work herein reported is being continued with particular reference to the morphological and pathological basis of low specific gravity in the seed and to the construction and use of equipment for delinting and gravity-grading on a practical scale.

SUMMARY

Cottonseed were delinted with sulphuric acid and then suspended in water and divided into the fractions that sank and floated, respectively. Laboratory, greenhouse, and field experiments on germination and emergence indicate that nearly all internally infected and weak seed are removed by this method. Healthy germination and emergence of the heavy seed was over twice as great as that of the light seed and half again as great as that of ungraded delinted seed. Practical application of the method appears feasible.

INCUBATION PERIOD OF PEA VIRUS 1 IN THE APHID MACROSIPHUM SOLANIFOLII

H. T. OSBORN

(Accepted for publication June 24, 1938)

In a previous paper[1] it was shown that both the pea aphid, *Macrosiphum pisi* Kaltenbach, and the potato aphid, *M. solanifolii* Ashmead (*M. gei* Koch), are vectors of pea virus 1. Transmission of pea virus 1 by the pea aphid is of considerable interest, because it is necessary for the virus to undergo an incubation period of about 12 hours before it can be transmitted by the insect and also because the aphid retains the ability to transmit the disease for long periods of time. When the paper was published, little was known about transmission of pea virus 1 by the potato aphid. Recent experiments have shown, however, that the virus undergoes a similar

[4] Communicated by Miss Tennyson and cited with her permission.

[5] TAUBENHAUS, J. J., and A. L. BURKETT. Control of damping-off of cotton seedlings. Texas Agr. Exp. Sta. Rep. 49: 105. 1936; and Germination and yields from acid-delinted cotton seed. *Ibid.* 49: 109. 1936.

[1] Osborn, H. T. Incubation period of pea mosaic in the aphid, *Macrosiphum pisi*. Phytopath. 25: 160–177. 1935.

period of incubation in the potato aphid and that it is retained in this aphid for a considerable period of time. Further tests were made also with the bean aphid, *Aphis rumicis* L. It is the purpose of this paper to present the data from these experiments.

MATERIALS AND METHODS

The virus used in most of the experiments was the same strain of pea virus 1 that was employed in studies on transmission by the pea aphid. It had been maintained in a greenhouse for a period of 6 years by successive transfers to fresh plants by means of the pea or potato aphids. In a few experiments another strain of pea virus 1 was used. This was obtained originally from pea aphids collected on pea plants growing near Princeton, New Jersey.

Colonies of the pink form of the potato aphid[2] were maintained in screened cages in a greenhouse on tomato, *Lycopersicon esculentum* Mill., Hubbard squash, *Cucurbita maxima* Duchesne, or potato, *Solanum tuberosum* L. The most vigorous colonies were produced on potato. Colonies of the bean aphid[2] were maintained on *Vicia faba* L.

In the experiments to be described, aphids from colonies on healthy plants were transferred by means of a camel-hair brush to diseased *Vicia faba* plants, where they were allowed to remain for periods of from 3 hours to 2 days. They were then transferred to a succession of healthy *V. faba* plants which were grown in 2½-inch pots. Immediately after exposure, the healthy plants were fumigated with Nicofume and returned to a greenhouse where they were grown beside noninoculated control plants.

INCUBATION PERIOD IN THE POTATO APHID

To determine whether or not an incubation period of pea virus 1 in the potato aphid is necessary for transmission, experiments similar to those previously reported for the pea aphid were undertaken. Although potato aphids ordinarily do not colonize on *Vicia faba* or peas, they feed readily when transferred to these hosts and, in previous experiments, had proved efficient carriers of the virus when fed for a day on diseased *V. faba* plants before transfer to healthy plants. They were found to be more easily injured than pea aphids. When transferred frequently at short intervals, the size of the colonies rapidly diminished. For this reason the results of several experiments were unsatisfactory because only a few of the plants exposed became diseased.

In one experiment, however, 5 colonies, each consisting of 75 potato aphids, were fed for 3 hours on diseased plants. They were then transferred to a succession of healthy plants at intervals of 3 hours until 9 sets of plants had been exposed. The 10th period of exposure continued for 17 hours and the final period for 3 days. The temperature of the green-

[2] Identification of aphids from these colonies was verified by Dr. P. W. Mason, Division of Insect Identification, U. S. Bureau of Entomology and Plant Quarantine.

house varied from 74° to 84° F. during this period. At the 10th transfer there were only 13 aphids in the 5 colonies. All were dead at the end of the final period. The results of the experiment are shown in table 1. Two

TABLE 1.—*Infections obtained with 5 colonies of the potato aphid when fed for 3 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Length of successive exposure periods (hours)	Approximate number of aphids on each plant	Succession of healthy plants				
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
3	75	–	–	–	–	–
3		–[b]	–	–	–	–
3		–	–	–	–	–
3		–	–	–	–	–
3		–	–	–	–	–
3		–	–	–	+	+
3		+[a]	–	–	–	–
3		–	–	–	–	–
3	13	–	–	–	–	+
17		–	–	–	+	+
72		–	–	–	–	+

[a] + = plant became diseased.
[b] – = plant remained healthy.

colonies became infective at the 5th period of exposure and one colony at the 6th, thus demonstrating an incubation period of not less than 12 nor more than 18 hours in 2 of the colonies and of not less than 15 nor more than 21 hours in a 3rd colony. Failure to infect consistently in subsequent periods of exposure is believed to have been due mainly to loss of infective individuals in the colony. Two colonies apparently failed to acquire the virus during a feeding period of 3 hours on diseased plants.

In a final experiment, potato aphids from a vigorous colony that had been reared on potato were used as vectors. Large-size nymphs and adults were fed for 4 hours on 8 *Vicia faba* plants showing severe symptoms of disease. At the end of this period the aphids were removed and transferred to a succession of fresh plants. Six sets of fresh plants were exposed at intervals of 4 hours. The aphids were then fed for 20 hours on potato. On the second day a fresh set of 8 *V. faba* was exposed for 4 hours. The aphids were then again fed on potato for 20 hours, and on the third day a final set of 8 fresh *V. faba* plants was exposed. A temperature of 74° to 78° F. was maintained throughout the experiment. Approximately 100 aphids were placed on each of the healthy plants during the first period of exposure. By the 4th period the colonies had been reduced to an average of 80 aphids, and by the end of the 6th period to an average of 55 aphids. At the end of the 8th period there were still approximately 50 aphids in each colony.

The infections obtained in this experiment are shown in table 2. No infection occurred in any of the plants exposed during the first 3 periods.

TABLE 2.—*Infections obtained with potato aphids when fed for 4 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Length of successive exposure periods (hours)	Approximate number of aphids on each plant	Number of plants exposed	Number of plants that became diseased
4	100	8 *V. faba*	0
4		"	0
4		"	0
4	80	"	2
4		"	3
4	55	"	3
20		Insusceptible potato	0
4	50	8 *V. faba*	6
20		Insusceptible potato	0
4		8 *V. faba*	5

Two plants were infected at the 4th period, 3 at the 5th period, and 3 at the 6th period. Six of 8 plants were infected when exposed for 4 hours on the 2nd day and 5 of 8 when exposed for 4 hours on the 3rd day.

The experiment demonstrated a minimum incubation period of not less than 12 nor more than 20 hours for some of the aphids in the colony. It also demonstrated retention of the virus by the colony for a period of at least 3 days.

RETENTION OF VIRUS BY APHIDS WHEN FED ON INSUSCEPTIBLE PLANTS

To determine whether the virus is retained by the aphids for periods longer than 3 days, they were fed on diseased *Vicia faba* plants for 1 day or more, transferred to tomato for a suitable period of time, and then returned to healthy *V. faba* plants. Tomato plants were used in these experiments, because they serve as satisfactory food plants for the potato aphid and at the same time are immune from infection by pea virus 1.

In one experiment approximately 500 large-sized nymphs and adults of the potato aphid that had fed on diseased *Vicia faba* plants for 24 hours were transferred to 4 small tomato plants. Transfers to fresh tomato plants were made at intervals of several days in order to remove newborn nymphs. After 14 days, the colony had dwindled to 100 individuals. These were tested for retention of the virus by transfer to 5 fresh *V. faba* plants for a period of 2 days. They were then fed for 4 days on tomato plants before being tested again by placing them on healthy *V. faba* plants for 2 days. Only 32 individuals were alive at the beginning of the final test. Of the 5 plants exposed to 100 potato aphids that had fed on insusceptible tomato plants for 14 days, all became diseased. Of the 5 *V. faba* plants exposed to 32 aphids in the final test, 4 became diseased. The infections obtained in this experiment demonstrated that the potato aphid retained the virus during a feeding period of 14 days on insusceptible tomato plants and during a total feeding period of 20 days.

In another experiment, potato aphids were fed for 2 days on *Vicia faba* plants diseased with the strain of pea virus 1 obtained near Princeton,

New Jersey. On removal from the diseased plant, 75 of the aphids were transferred to 5 healthy *V. faba* plants, while 350 were placed on tomato plants. The aphids on tomato plants were transferred at intervals of several days in order to destroy newborn nymphs. After a feeding period of 14 days on tomato, 75 aphids were removed and transferred to 5 small *V. faba* plants. After 21 days, only 27 aphids remained on the tomato plants and these appeared inactive and weak. The 27 aphids were removed from tomato at this time and placed on 5 small *V. faba* plants. Of the 5 *V. faba* plants exposed to 75 potato aphids immediately after removal from diseased plants, all became infected. Of the 5 plants exposed to 75 aphids that had fed on tomato plants for a period of 14 days, all became infected, while of the 5 *V. faba* plants exposed to 27 aphids that had fed on tomato for 21 days, one became infected. This experiment demonstrates that potato aphids, having once acquired the virus, may retain it during a continuous feeding period of 21 days on insusceptible tomato plants.

EXPERIMENTS WITH THE BEAN APHID

In previous experiments[3] the bean aphid failed to transmit pea virus 1, but did prove to be an efficient vector of pea virus 2.[4] Since the aphid feeds readily and multiplies rapidly on *Vicia faba*, it seemed desirable to conduct further tests to determine whether or not it might occasionally transmit pea virus 1.

For this purpose bean aphids were colonized on diseased *Vicia faba* plants for 4 or 5 days. Heavily infested leaves were then cut off and placed beside small healthy plants so that the aphids migrated naturally from the diseased to the healthy plants. In one series 50 small *V. faba* plants were exposed to aphids that had fed on *V. faba* plants diseased with the strain of virus from New York. In another series 100 small plants were exposed to a strain of the virus secured near Princeton, New Jersey. Of the 150 small plants exposed, not one developed symptoms of pea virus 1. This result confirms previous experiments and demonstrates conclusively the inability of the bean aphid to transmit this virus.

DISCUSSION

In nature, the pea aphid undoubtedly is the principal vector of pea virus 1, since it occurs in abundance on legumes susceptible to the virus. The potato aphid, also capable of carrying the virus, commonly occurs on insusceptible solanaceous hosts and for this reason is probably not an important vector in the field. It seemed of interest, nevertheless, to determine the mode of transmission of pea virus 1 by the potato aphid, as well as by the pea aphid, because an incubation period has been shown for only one other virus transmitted by aphids. Potato-leaf-roll virus undergoes

[3] *Loc. cit.*

[4] Osborn, H. T. Studies on the transmission of pea virus 2 by aphids. Phytopath. 27: 589–603. 1937.

an incubation period in the green peach aphid, *Myzus persicae* Sulzer.[5,6] The results of the experiments show definitely that transmission of pea virus 1 by the potato aphid involves an incubation period and that the virus is retained by the aphids in a manner similar to that previously shown for the pea aphid.

SUMMARY

By exposing successions of healthy *Vicia faba* plants to colonies that had fed for short periods on diseased plants, it was shown that pea virus 1 undergoes an incubation period in the potato aphid, *Macrosiphum solanifolii*. The minimum incubation period demonstrated was not less than 12 nor more than 18 hours.

Potato aphids that acquired the virus were shown to retain it for as long as 21 days when fed continuously on insusceptible tomato plants.

The bean aphid, *Aphis rumicis*, failed to transmit the virus.

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PHYTOPHTHORA ROT OF ASPARAGUS IN CALIFORNIA

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(Accepted for publication July 15, 1938)

In the middle of March the attention of the writers was called to a soft rot in asparagus shipped from Valdez, California, to Chicago and New York.

The examination of asparagus spears that were delivered to the packing house from nearby fields showed definite lesions on the stem. These lesions were in the form of spots of different sizes, water-soaked and soft (Fig. 1). This type of asparagus spear was usually discarded by workers before bundling the asparagus preliminary to packing and precooling. Loose asparagus, as well as bundled, was subjected to laboratory study. Spears with definite lesions were cultured. The cultured spears yielded pure cultures of *Phytophthora* sp. that, upon inoculation into healthy asparagus, produced infection similar to that observed on cultured spears. Upon reculturing, a pure culture of *Phytophthora* sp. was isolated in all particulars similar to the one introduced. The *Phytophthora* sp. was isolated from asparagus from the above-mentioned localities by C. M. Tompkins of this Division in March, 1936, and recently reported[1] to be found in the New York markets on asparagus from this area. The *Phytophthora* sp. causing this

[5] Elze, D. L. De verspreiding van virusziekten van de aardappel (*Solanum tuberosum* L.) door insekten. Meded. Inst. Phytopath. Lab. Mycol. Aardappel-onderzoek, Wageningen 32: 1927.

[6] Smith, K. M. Studies on potato virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. Am. Appl. Biol. 18: 141–157. 1931.

[1] Wiant, James S., and C. O. Bratley. Diseases of fruit and vegetables on the New York market during January, February, March, and April, 1938. Plant Dis. Reptr. 22 (11): 190–193. 1938.

disease in asparagus is being studied by the junior writer and an account of it will be published soon.

On field material an abundance of oospores and sporangia was observed and discharge of zoospores occurred when specimens were placed in water. When the tips of asparagus are affected by the *Phytophthora* sp. a softening of this part of the plant is developed, which, being accompanied by saprophytic bacteria, produces an extremely vile odor. The bacteria accompanying the Phytophthora rot were not found capable of attacking the healthy tips when inoculations were made with pure cultures.

To ascertain the degree of infection in asparagus that was bundled and ready for precooling, bundles of asparagus spears were brought to the laboratory and stored at 41° F. for a period of 5 days, after which a count of

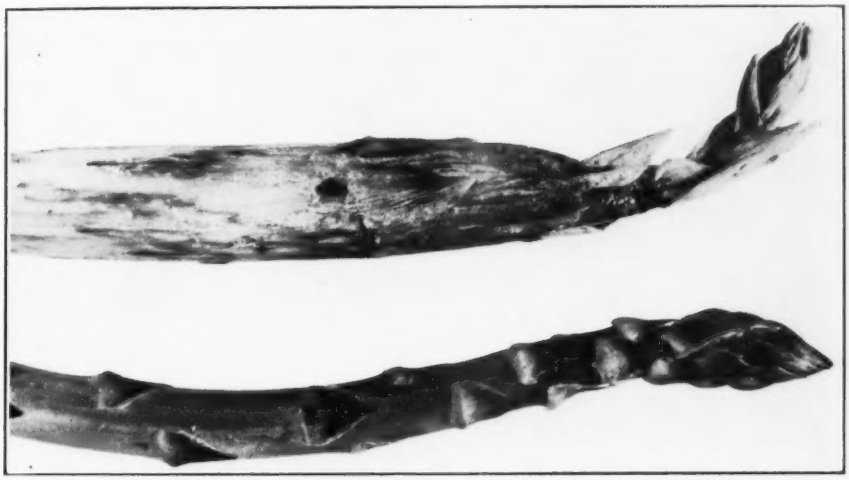

FIG. 1. Stalk rot of asparagus caused by *Phytophthora* sp. Naturally infected stalk of French Argentile variety on top, healthy stalk at bottom.

diseased spears was taken. A representative number of diseased spears was cultured to be sure the symptoms observed were produced by *Phytophthora* sp. The tests showed that the infection ran from 6 to 18.5 per cent.

Similar conditions existed in a packing house at Antioch, California. Through the courtesy of Mr. Lewis Lawyer several bundles of asparagus were obtained and, upon examination, were found to contain from 20 to 30 per cent infected spears after 5 days' incubation at 41° F. These tests clearly indicated, (a) that the Phytophthora rot was present in the field and (b) that in spite of inspection prior to the bundling, infected spears escaped the inspector's notice. Furthermore, it was important to establish if the *Phytophthora* sp. was present in the water used in the precooling operation. The water samples collected after a day's run of the precooler, when cultured, yielded numerous colonies of the *Phytophthora*. It was not shown, however, that the practice of precooling in water had any pronounced influence on the ultimate amount of disease.

This situation raised the pertinent question of the possibility of water disinfection with the simultaneous disinfection of the asparagus spears that might have either the early stages of the disease or the zoospores lodged on them. With this idea in mind a number of disinfectants was tried out under laboratory conditions. The chemicals were tested on the fungus and also on asparagus spears. To test toxicity of the selected chemicals the fungus was grown on a potato-dextrose-agar plate and then cut into small discs by means of a sterilized cork borer. These bits of fungus were dropped into the test solutions for various intervals of time and, after washing in sterile distilled water, were placed into potato-dextrose-agar plates with subsequent incubation at room temperature. These tests showed that *Phytophthora* sp. was killed as follows: by 2 per cent of Labarraques solution (2.6 per cent sodium hypochlorite) in 15 minutes; by $\frac{1}{4}$ per cent of commercial ammonium hydroxide (28 per cent strength) in 10 minutes; by $\frac{1}{4}$ per cent of sodium peroxide in 5 minutes; by $\frac{1}{2}$ per cent sodium peroxide in $\frac{1}{2}$ minute; by 0.05 per cent sodium peroxide in 5 minutes. The organism was killed in one minute by water at 46° C. The test showed no injury to tender asparagus spears by the above chemicals and hot water for the duration of the experiments. Treatments of asparagus with field infections, as well as asparagus artificially inoculated in the laboratory by dipping into a water suspension of the zoospores, showed a considerable efficacy in checking the trouble under the experimental conditions.

The disease appeared to be related to a period of heavy and prolonged rainfall and perhaps to the rather common practice of flooding certain areas to produce earlier growth.

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PHYTOPATHOLOGICAL NOTES

Nematode Infestation of Olive Roots.—For the past several years it has become increasingly evident that fig trees in California are seriously affected by a species of nematode[1] different from that causing the well-known root-knot or bead-like swellings of the fibrous roots. The former infests the bark of the larger roots, resulting in necrotic areas and often in complete destruction of the affected root, especially of the taproot.

Recently, the writers had occasion to observe the roots of 10-year-old olive trees that were being removed. These alternate trees in fertilizer plots at this Station were apparently normal and healthy as to the tops. The roots of many, however, showed an abnormal condition manifested by longitudinal cracks in the bark, some being black and necrotic, others whitish and callused. Specimens were sent to Dr. G. Steiner, United States Department of Agriculture, Washington, D. C., who reported that the "roots

[1] Gerald Thorne. Some plant parasitic nemas, with descriptions of three new species. Jour. Agr. Res. [U. S.] 49: 755–763. 1934.

are infested with the same nematode species previously reported from the fig tree roots which you sent in. In our conception, the species is *Pratylenchus musicola* and not *Pratylenchus pratensis* as reported by Thorne," the differentiating characters of the two species having been very vague in the past.

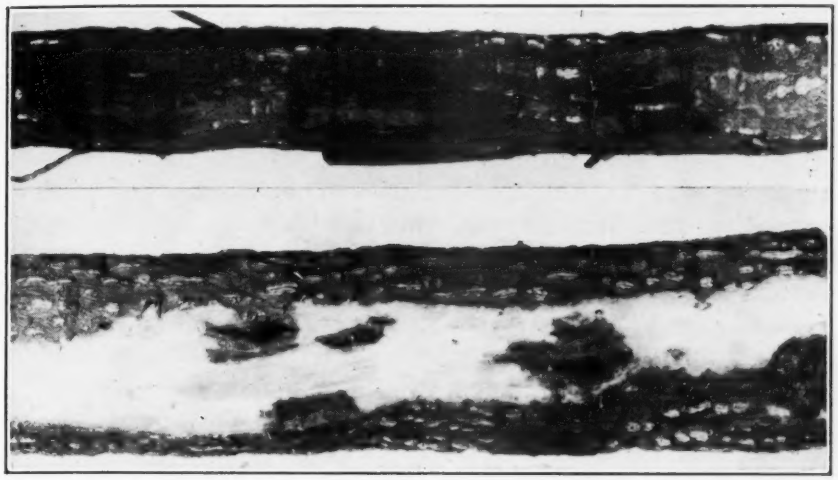

FIG. 1. Roots of olive trees at the Citrus Experiment Station, Riverside, California, showing lesions in which the nematode, *Pratylenchus musicola*, occurs abundantly.

This nematode apparently attacks only the bark portion of the olive root and does not kill the cambium nor cause a rotting of the larger roots, as it does in figure 1. Until further observations are made on its prevalence in olive districts, we are unable to state what effect infested roots may finally have upon the nutrition and productiveness of this new host plant.—IRA J. CONDIT AND W. T. HORNE, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

A Phloem Necrosis of Elm.—For several years an epidemic dying of American elms has occurred in the central and lower Ohio River watershed. In Ohio it was first reported at Ironton in 1918 and at Dayton in 1927. Since then a similar condition has obtained in many localities throughout the region. This dying was probably prevalent in southwestern Indiana and northern Kentucky before it was discovered in Ohio. It is impossible at present to determine whether all these epidemics have been due to the same cause.

In Chillicothe, Ohio, an epidemic of this type began in 1935. In the spring of 1936 an investigation was begun there and elsewhere. A thousand trees, 50 per cent of the city's elms, died in 1936 and 1937. Affected trees died in from 3 to 36 months after the first apparent symptoms. No recovery was observed. The disease is extremely virulent and has spread rapidly in Ohio on the American elm.

Symptoms are first noticeable in the extreme top at outer tips of branches. The foliage becomes thin, the leaves droop, because of downward curvature of the petioles. The leaf blade curls upward at margin, producing a trough-like effect that makes the leaves appear narrow and greyish-green and entire crown of tree thin. Such leaves are often stiff and brittle. Later, foliage becomes yellowish-green and finally yellow, followed by defoliation. These symptoms usually occur throughout the entire crown; are not confined to one or several branches, as is frequent with common wilts; there is no vascular discoloration.

In fairly advanced stages of the disease the roots die, the small fibrous ones first. Typical discoloration, confined to phloem and cambium, precedes death of larger roots and may be found frequently extending into trunk and branches. In large trees this discoloration usually is found in large roots and at base of trunk, just before death. The cambium first becomes light yellow or golden. The phloem becomes yellow in region adjacent to cambium, then brown, with small, scattered, black flecks. Soon thereafter, phloem becomes dark brown and necrotic. An odor resembling wintergreen characterizes moderately discolored phloem.

Over 4,000 attempted plate isolations, averaging 10 plantings each, have been made from the roots, trunks, and branches of over 400 diseased trees. No organism has been secured consistently; none appears associated with the disease. Over a 2-year period inoculations with the organisms obtained have failed to indicate their pathogenicity.

Histological study of diseased tissue has shown no organism consistently present. In almost all cases the organisms found were confined to the dead roots.

Direct insertion of diseased tissues into 72 healthy elms has not resulted in transmission.

Healthy elms with injured roots planted in soil secured from around diseased trees in the field remained healthy. Planting healthy elms with artificially injured roots in a compost consisting of 50 per cent diseased roots and 50 per cent soil by weight resulted in no transmission.

In July, 1937, 21 healthy American elms were grafted with patches of bark from diseased trees, the patch showing typical phloem discoloration. Five nongrafted trees were left at random as checks. In August, 1938, the disease was apparent in 14 grafted trees, all showing complete symptom expression. The nongrafted check trees in this block and approximately 400 other elms in the nursery remained healthy.

In January, 1938, 20 healthy trees were grafted with branch scions from diseased trees, and the roots of 26 healthy elms were grafted with diseased root scions. Four healthy trees were grafted with healthy branch scions and 4 with healthy root scions as checks. These trees were planted in the greenhouse. Of the branch-grafted trees, union between scion and stock occurred on the 4 check trees and on 15 of those grafted with diseased scions. Up to September 12, 1938, all checks and trees unsuccessfully grafted remained

healthy. Of the remaining 15 trees, transmission and complete symptom expression were obtained on 13.

Up to September 12, 1938, transmission and complete symptom expression were obtained on 5 trees grafted with diseased root scions. All check trees remained healthy. Whether union between diseased root scion and healthy stock was secured on the remaining trees can not be determined until the trees are dug after they are dormant.

From the experiments undertaken, it appears that this disease is of a virus nature and that it is systemic. How it is transmitted under natural field conditions is yet unknown.—ROGER U. SWINGLE, Division of Forest Pathology, U. S. Department of Agriculture, in cooperation with the Ohio Agricultural Experiment Station, Wooster, Ohio.

Preliminary Tests to Determine Effect of Arsenite Sprays on Sporodochia of Sclerotinia laxa and on Control of Brown Rot in Blossoms of Almond and Apricot.—By spraying apple leaves in the autumn with calcium and zinc arsenite in combination with copper sulphate and lime, Keitt and Palmiter[1] prevented the development of ascospores by *Venturia inaequalis* (Cke.) Wint. They were able also to suppress the production of primary inoculum by other fungi.

The present writers wish to report the results obtained by spraying almond and apricot trees with arsenite sprays for control of the brown rot caused by *Sclerotinia laxa*. The sporodochia are the major if not the only source of primary inoculum for blossom infection in these crops. They are produced abundantly during spring on mummied fruit and on twigs blighted the previous year.[2]

In 1936, apricot trees sprayed with a sodium arsenite solution at the time blossoms were emerging from the bud (after sporodochia had appeared), developed markedly less blossom infection than nonsprayed trees. In 1937 apricot trees again were sprayed in the same stage with various concentrations of sodium arsenite and with monocalcium arsenite (4 pounds to 100 gallons). As trees in this orchard failed to develop blossoms, no observations on incidence of the disease could be made.

In 1938 monocalcium arsenite, zinc arsenite, and sodium arsenite were applied to almond and apricot trees. As the purposes of these tests were to determine the effect of the sprays on the development of sporodochia, on germinability of sporodochium conidia, and on subsequent development of blossom infection, the applications were made both before and after sporodochia appeared in the trees.

Sodium arsenite (1.2 pounds to 100 gallons of water), applied to almond trees before sporodochia appeared, had no apparent effect on their development, and little, if any, effect on development of blossom infection. This material (1–100), however, applied to apricot trees after sporodochia ap-

[1] Keitt, G. W. and D. H. Palmiter. Potentialities of eradicant fungicides for combating apple scab and some other plant diseases. Jour. Agr. Res. **55**: 397–437. 1937.

[2] According to unpublished data by Wm. B. Hewitt and L. D. Leach.

peared, markedly reduced germinability of the conidia and prevented blossom infection to a considerable extent.

Monocalcium arsenite (4–100) plus 4 per cent of a dormant type petroleum oil emulsion, applied to almond trees before sporodochia appeared, suppressed sporodochium development 96 per cent and reduced the incidence of blossom infection 80 per cent. When applied after sporodochia had appeared, this material reduced germinability of conidia 97 per cent and the incidence of blossom infection 71 per cent.

Zinc arsenite (4–100) with or without 4 per cent oil emulsion was not so effective in preventing sporodochium development. When applied after these structures appeared, however, zinc arsenite (3–100) plus 2 per cent oil emulsion, reduced germinability of conidia 90 per cent and the incidence of blossom infection 83 per cent.

Although too meagre for final conclusion, these results are sufficiently promising to warrant further tests. Much is yet to be done in determining the effect of the materials on the tree.—E. E. WILSON and E. F. SERR, Division of Plant Pathology and Agricultural Extension Service, respectively, University of California, Berkeley, California.

Inoculation of Conifers with the Cypress Coryneum.—A canker caused by *Coryneum cardinale* has been reported by Wagener[1, 2] as attacking and eventually killing trees of Monterey cypress, *Cupressus macrocarpa*.[3] This disease also can attack other species of *Cupressus*[2] but to a lesser degree. It has reduced a planting of *C. macrocarpa* on the campus of the Citrus Experiment Station to a few trees and these are now severely attacked. *Cupressus sempervirens* and *C. glauca* (*C. arizonica, var. bonita*) growing near have apparently escaped.

Inoculations were made through wounds made in the bark of small trees by means of a 4 mm. cork-hole borer. Two methods of inoculation were used: (1) Placing the mycelium of a pure culture in the wound, and tying over it some moist absorbent cotton. Then the whole is wrapped with paraffin paper, with the ends fastened to the branch with a band of nurseryman's tape. This method would retain the moisture and prevent for some time desiccation of the mycelium. (2) The second method involves substituting for the mycelium disks cut from a diseased cypress lesion on which the fungus is fruiting. These disks were placed in the wounds with the spore-bearing surface in contact with the tissue. Nurseryman's tape was used to wrap these inoculation wounds. One inoculation and one control were used in each test. After the lapse of 2 months, data were taken on the inoculations. It should be stated that according to the temperature records during this time, from January 27 to March 30, 1938, the maximum temperature in Riverside had not exceeded

[1] Wagener, W. W. Coryneum canker of cypress. Science (n.s.) **67**: 584. 1928.

[2] Wagener, W. W. The cypress bark canker and other cypress diseases. Third Western Shade Tree Conf. Proc.: 79–85. 1936.

[3] Cypress bark canker and the Monterey cypress. Science (n.s.) **86**: 2230. Suppl. 8–9. 1937.

81° F. It was a time of moderate temperature with no excessively hot and drying periods. The trees were growing in 5-gallon cans in a lath house.

The positive results of these inoculations are summarized in tables 1 and 2. The sources from which the trees were obtained are indicated. The names

TABLE 1.—*Lesions on species of Cupressus from inoculations by Coryneum from cypress canker: 1 inoculation and 1 control in a series. S = Accession number of Rancho Santa Ana Botanic Garden. SPI = U. S. Department of Agriculture Seed and Plant Introduction number. Nursery indicates purchased from a local nursery. CES = Tree on the Citrus Experiment Station grounds. TL = Seed came from type locality*

Host species	Source of hosts	Diameter of stem inoculated	Radius of lesion after 2 mo.	Length of lesion after 4 mo.	Amount of stem girdled
		mm.	*mm.*	*mm.*	
arizonica	S 2357	10	20	60	½
arizonica	S 2358	12	10	50	½
arizonica	Seedlings	15–17	10–15–20	15–30	¼–⅞
bakeri TL	S 2127	10	15	90	all
duttoni TL	S 2157	12	15	35	½
forbesi	S 2315	15	20	45	⅝
forbesi	S 2319	15	15	60	½
forbesi TL	S 2335	10	15	45	⅝
glabra	CES	20	30	70	½
goveniana	S 2181	12	30	60	all
goveniana TL	S 2182	14	20	50	all
guadalupensis	Nursery	20	15	30	½
lusitanica	SPI 73844	30	30	90	½
macnabiana	S 2154	10	15	30	all
macnabiana	S 2118	10	30	45	all
macrocarpa	S 2177	6	30	50	all
macrocarpa TL	S 2184	15	30	70	all
pygmaea TL	S 2133	15	25	70	½
pygmaea	S 2137	11	35	78	¾
sargenti	S 2185	15	20	60	⅞
sargenti	S 2167	7	20	90	all
sargenti TL	S 2156	10	20	70	all
sempervirens	CES	12	25	40	½
thurifera	S 2356	17	22	70	⅝

TABLE 2.—*Lesion produced 2 months after inoculation on species of the Cupressaceae by Coryneum from cypress: 1 inoculation and 1 control in a series. Nursery = Purchase from local nursery. SPI = U. S. Department of Agriculture Seed and Plant Introduction number. CES = Tree on campus of Citrus Experiment Station*

Host species	Source of hosts	Maximum radius of lesion in mm.
Thuja plicata	Nursery	15
T. orientalis	CES	30
T. occidentalis	Nursery	20
Juniperus cedrus	SPI 57080	20
J. californica	CES	20
J. phoenicea	SPI 65020	0
J. procera	SPI 27505	0
J. procera	SPI 60553	0
J. virginiana	Nursery	10
Libocedrus decurrens	"	10
Chamaecyparis lawsoniana	CES	0

and identification number are those of the institution from which the material was received. An examination of the inoculations on *Cupressus* spp. after 3.5 months (1.5 months, since taking the data listed in Table 1) shows a marked advance of the lesions around the inoculated stems, which are now $\frac{1}{3}$ to $\frac{3}{4}$ girdled, with a few stems entirely so. The spread of the disease lesion on the other inoculated species of the family Cupressaceae has not been so rapid. The cypress *Coryneum* is apparently parasitic through wounds on many species of a number of genera of the family Cupressaceae (*Cupressus*, *Juniperus*, and *Thuja*). It probably is true that, under natural conditions, some of these species may not prove to be susceptible to the disease.

Inoculations in the following species of conifers in these experiments gave negative results: *Pseudotsuga taxifolia*, *Taxus baccata*, *Ginkgo biloba*, *Cryptomeria elegans*, *C. japonica*, *Araucaria bidwilli*, *Pinus halepensis*, *Taxodium mucronatum*, *Sequoia gigantea*, *S. sempervirens*, *Larix europea*, and *L. leptolepsis*.

Inoculations made on rose, peach, oak (*Quercus lobata*), elm (*Ulmus parvifolia*), and walnut (*Juglans regia*) gave negative results.—CLAYTON O. SMITH, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

A Technique for Studying the Longevity of Phoma lingam in the Soil.—When visiting the University of California in 1936, I learned from Dr. P. A. Ark of his technique for detecting the presence of *Bacillus amylovorus* in soils. Injured immature pears were suspended for 15 minutes in an aqueous suspension of the suspected soil. If viable organisms were present fire blight developed on the pears.

On my return to New Zealand, experiments were made to ascertain whether the method could be used to demonstrate the presence of *Phoma lingam* in soil. Roots of Superlative swedes were topped, washed, and incised with a sterile scalpel. In groups of 4 they were immersed in one of a series of aqueous spore suspensions containing from one spore per 10 cc. to 100,000 spores per 1 cc. Dry-rot lesions, typical of those caused by *P. lingam*, developed on swedes steeped in suspensions containing 5 or more spores per cc. (Table 1 and Figure 1, A–E).

When infection was heavy (as was caused by the suspension containing 100,000 spores per cc.) fructifications of the fungus appeared on swedes within 12 days. Normally *P. lingam* is slow to fruit, 6 weeks being required when inoculations are made from agar cultures on swedes, and 2 months on potato-dextrose agar cultures incubated at 21° C.

To test the application of the method in the field, aqueous suspensions were made with soil collected within a foot of infected swedes and also with soil taken from the same places 2 months after the removal of the swedes. Roots steeped in both suspensions became heavily infected with *Phoma lingam* (Fig. 1, F).

TABLE 1.—*Dry-rot infection of swedes 10 days after submergence in aqueous suspensions of spores*

Strength of suspension	Number of wounds on 4 roots		Percentage of wounds infected
	Infected	Healthy	
0.1 spore per cc.	0	58	0
5 spores " "	2	71	3
50 " " "	44	35	56
100 " " "	24	49	33
500 " " "	56	3	93
1000 " " "	55	7	89
10,000 " " "	48	5	91
100,000 " " "	55	0	100
Check	0	62	0
Steeped in soil suspension	42	3	93

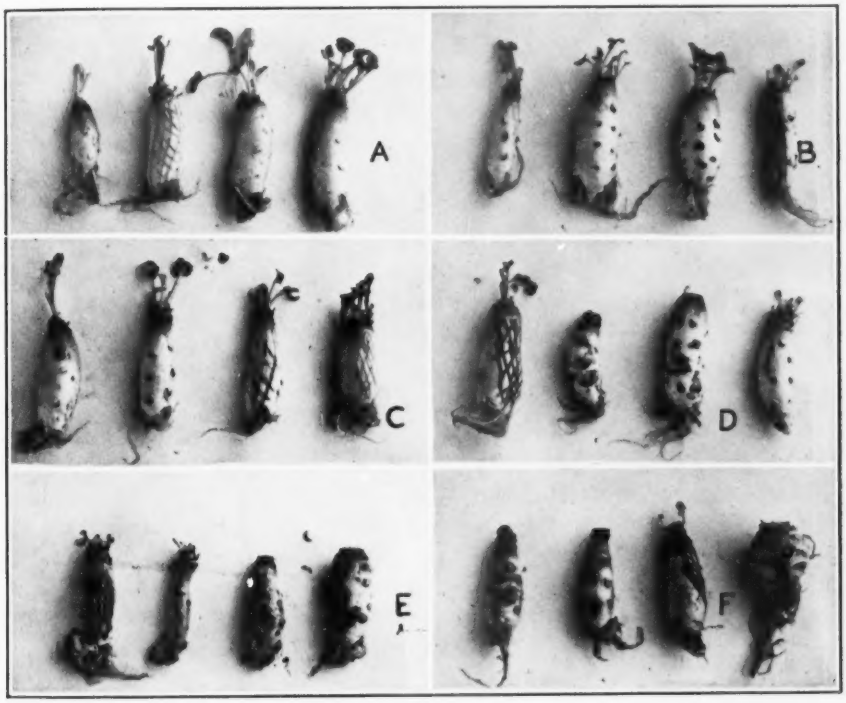

FIG. 1. Injured swedes. A. Check steeped in sterile water. B–E. Steeped in suspensions of *Phoma lingam* spores containing respectively: B, 50 spores per cc.; C, 100 spores per cc.; D, 500 spores per cc.; E, 100,000 spores per cc. F. Steeped in soil suspension.

The method facilitates studies on the longevity of *Phoma lingam* in the soil, a subject previously proved difficult owing to the numerous contaminations that occur in dilution plates and the slow growth of the fungus in culture.—J. G. GIBBS, Plant Research Bureau, New Zealand Department of Scientific and Industrial Research, Palmerston North, New Zealand.

The Longevity of Ceratostomella ulmi in Soils.—In the eradication program against the Dutch elm disease, the question of the growth and survival

of the causal fungus, *Ceratostomella ulmi* (Schwarz) Buisman, in soils was raised as one that might have a decided influence on the program. Infected roots and stumps in many cases had to be left when diseased trees were destroyed, and, if the causal organism spread from these and other sources into the soil and survived for any considerable time, the control program might be seriously affected.

Attempts were made to isolate *Ceratostomella ulmi* from soils around diseased stumps in nature. Many of these attempts were made from soil in contact with diseased wood from which *C. ulmi* was isolated at the time of soil sampling. Soil samples taken near the root collar were in contact with debarked wood, while those taken near small lateral roots were in contact with the bark only that in some cases was alive and in others dead. Eight stumps under various conditions of soil and exposure were used and soil cultures were made several times during the spring of 1936. No *C. ulmi* was isolated from any of these soil samples.

In order to get better sampling, 6 live, diseased stumps of naturally infected trees were placed, in the spring of 1936, in a sandy loam soil in 6 concrete bins constructed with drains for collecting run-off water. The soil was kept moist by artificial watering when necessary. After the stumps had been in place 30 to 60 days, sufficient water was added to secure a liter of run-off from each bin. No *Ceratostomella ulmi* was isolated from the water. In another bin heavily infected elm stem wood was buried, and a small amount of *C. ulmi* was isolated from run-off collected after the wood had been buried for 14 days. No further isolations were attempted.

In September, 1935, 7 lots of 10 test tubes each, approximately 2 inches in diameter and 16 inches long, were partly filled, respectively, with clay loam, loam, clay, sand, swamp muck, hardwood-forest humus, and forest litter, all from the Dutch elm disease area in New Jersey. Five of the tubes of each soil type were sterilized on each of 3 consecutive days at 105°–110° C. All were then inoculated with a heavy suspension of spores of *Ceratostomella ulmi* and incubated under laboratory conditions. Throughout the experiment the tubes were kept at a constant weight by adding sterile distilled water when needed to maintain the soils in a well-moistened condition.

After 3 months, *Ceratostomella ulmi* was isolated in abundance from all the sterilized soil samples, but it was not isolated from any of the nonsterilized soils, except the forest humus and only sparingly from that. After 6 months no *C. ulmi* was isolated from the nonsterilized series, but it was recovered from the sterilized soils. At that time all the nonsterilized soils were reinoculated with *C. ulmi*, which could not be reisolated 4 months later. By that time (10 months after the original inoculations) the sterilized soils had become contaminated and *C. ulmi* was isolated from but 10 of the 35 tubes.

In another test about 30 samples each of sand, clay, rich forest humus, and elm leaves were placed in 50-cc. beakers. One-third of the samples of each soil type were steam-sterilized for 20 minutes at 15 pounds' pressure. Distilled water was added to each beaker to make 2 series of moisture contents, one for sterilized and the other for nonsterilized soils, varying for each soil

type from air-dry to supersaturated. All were then inoculated with a heavy spore suspension of *Ceratostomella ulmi*. The air-dry samples were incubated over calcium chloride in desiccators. The other samples were incubated in "desiccators" in which a saturated atmosphere was maintained. The desiccators were all stored at room temperature. *C. ulmi* developed rapidly in the sterilized series, except in air-dry soils, and an abundance of conidia and coremia formed on the surface. A few coremia also formed on the nonsterilized clay, but all macroscopic evidence of *C. ulmi* disappeared after 30 days. Coremia were still abundant in the sterilized series after 120 days. Isolations made at that time showed the fungus to be alive in all except one of the sterilized samples, but it was recovered from only one beaker of sand stored over calcium chloride in the nonsterilized samples.

When humus soil treated in 3 ways (sterilized, nonsterilized, or sterilized and subsequently reinoculated with a suspension of its previous organisms) was inoculated with *Ceratostomella ulmi*, the fungus survived only in the sterilized soils not reinoculated with soil organisms. This indicates that survival or lack of survival of *C. ulmi* in soil is dependent largely on the absence or presence of competing organisms.

In the experiments here reported isolations were made from soil suspensions poured on elm-wood chips, acidified malt or potato-sucrose agar, and potato-sucrose agar containing 3 cc. of 10 per cent of commercial formaldehyde to 400 cc. of agar. Tests have shown that if *Ceratostomella ulmi* is present, even in very small amounts, it can be detected in natural soils on at least one of the media used. Consequently, the data indicate that, even if *C. ulmi* passes into the soil, in nature, it will survive but a short period in competition with soil organisms. So far, *C. ulmi* has not been isolated from soil in nature.—A. F. VERRALL, Dutch Elm Disease Laboratory, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Morristown, N. J.

BOOK REVIEW

BOYCE, JOHN SHAW. *Forest Pathology*. 1st Ed. 600 pp., illus. McGraw-Hill Book Company, New York and London. 1938. $5.00.

The book devotes itself to the diseases of forests and forest products rather than of shade trees. In territory, it covers the United States and Canada; but full use is made of pertinent European material. It is designed, primarily, for use in teaching pathology to advanced forestry students, but the author has succeeded by economy of words and careful selection of literature in making the book also an exceptionally useful work of reference; there is much information that is useful only for reference. The degree of compression of the book, as also the magnitude of the field for which the forest pathologist is responsible, is indicated by the fact that there are considered diseases of more than 200 host species. The bibliography contains more than 1300 titles, placed at the ends of the chapters to which they apply and particularly featuring papers that themselves contain bibliographies. It is well indexed, 30 pages being so employed. The numerous good illustrations are closely pertinent to the text. Following general chapters on disease and on fungi, the diseases are classified partly on the basis of cause or type and partly on the age or part of the plant infected; one of the best indications of the practical workability of this classification is in the fact that most of the literature references have important bearing only on the material of the chapter to which they are attached. The group of subjects most fully covered in the book is the decays and fungus discolorations of wood, covering 30 per cent of the text matter—an emphasis reflecting both the importance of heart rots during the harvesting of the remains of our overmature forests, and the fact

that they are relatively well known, as well as the author's intimate acquaintance with them. A noteworthy feature is the chapter on the deterioration of dead timber. Other topics appropriately featured include seedling diseases, chestnut blight, white pine blister rust on which, also, the author, himself, is an authority, and Dutch elm disease.

Although generalized discussions for each type of disease appear at the beginning of each chapter, in addition to the brief introductory chapters, the reviewer feels that somewhat more material of a general nature and a slightly more philosophic approach would have been justified, even at the expense of still further compression of the information about the individual diseases. In the introductory chapters one misses the historical background of the subject. For the orientation of the forestry student in the pathology field it would seem desirable to include in them more consideration of resistance, predisposition, variation in strains of parasites, dissemination, insect relationships, and morbid anatomy and physiology. The emphasis of the discussional portions of the book is more economic than biological. The treatment is in accord with the concept developed by E. P. Meinecke, that forest pathology must concern itself more with the stand than with the individual tree; but this fundamental principle is nowhere definitely expressed in the book. Meinecke's ground-breaking paper on evaluation of damage is unfortunately referred to in connection with the specific rust with which it dealt rather than with the methodology of damage studies that it was primarily intended to illustrate. The contribution of disease to the uncertainty of forest enterprises might receive more attention. The various disease hazards that arise as a result of the increasingly artificial conditions in the forest, such, for instance, as the change in the central and northern hardwood stands from seed origin to sprout origin, could be better brought out. The reader will fail to get an adequate picture of the achievements and limitations of the remarkable campaign in control of white pine blister rust. General plant pathologists might find interesting a statement of the position to which creosote has advanced in the front rank of fungicides, and the part played by impregnation treatment of less durable woods in averting the exhaustion of durable woods and in keeping wood in the picture for poles and railway ties. Many of these points are considered in the final chapter on principles of control, but some expansion and redistribution of material of this sort would improve the book.

The consideration of wilts does not adequately bring out the concept originated by F. C. Craighead, of the damage associated with the bark beetles of the genus *Dendroctonus*, the most destructive group of insects in American forests, as being in fact apparenly due to fungi that are absolutely dependent on the beetles for transmission.

Not as a criticism, but rather as evidence of the rapidity with which new information is being secured on forest diseases, it is noted that the book is already out of date on a number of points because of publications that have appeared since the text was sent to the publisher. The forest-pathology field is still in somewhat the state of crop-plant pathology in the days when Halsted was issuing his numerous papers on new diseases. Perhaps one of the best indications of the incompleteness of the knowledge of forest-tree diseases and the amount of new material that must still be lying near the surface, is the fact that the author finds for inclusion only a single American disease of the virus type for which transmissibility has been demonstrated. To the conditions mentioned as suspected of virus origin, might be added bird's-eye maple.

The treatment is conservative. The author indulges in little speculation of his own, though statements or recommendations with little more than speculative basis have unavoidably been accepted from the literature. There are very few statements with which it is possible to definitely disagree. Excess soil moisture is stated to deprive roots of oxygen because they cannot take it from water; it would be more in accordance with our other information to suppose that water-logging of soil causes oxygen hunger by hindering the movement of oxygen into and through the soil. The statement concerning the eradication of building rots departs from the general limitation to thoroughly practicable measures, in calling for the removal of *all* infected wood. So uncompromising a recommendation is often impracticable and unnecessary if sources of moisture are cut off and sufficient ventilation is provided.

In the control chapter native forest diseases are described as controllable to a sufficient degree by a proper carrying out of the various thinning and cutting operations that are a usual part of intensive management. Special attention is given the problem of the introduced disease. For present protection against foreign parasites quarantine is recognized as the most effective measure, for the various other ways of combatting introduced parasites, which are available for crop plants, are difficult or impossible to apply under forest conditions. The need for more information on diseases whose introduction may be feared is properly emphasized, as requiring a study of American species planted abroad. It might be further pointed out that most of the danger is from Asiatic fungi, in view of the large number of Asiatic tree species related to ours, and that so few American trees have been planted in Asia that special plantings would have to be made there for disease observation.

The book, in general, is a highly valuable addition to the working equipment of both pathologists and foresters. At the same time it furnishes a good summary of accomplishments in a field that has received concentrated attention during only the last 30 years.—CARL HARTLEY, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

ANNOUNCEMENT

Abstracts of Plant Science. The trustees of BIOLOGICAL ABSTRACTS have adopted a new plan of publication, to begin in 1939 that is of more than passing interest to this Society. The emergency subsidy plan in effect in 1938 will be abandoned in favor of self-support. As heretofore, there will be monthly issues covering the whole life-science field. The aim is increasing promptness, a lag of not over two or three months between printed article and printed abstract, and the annual analytical subject index in the spring. In addition to the complete edition, the monthly issues will be divided up into separately bound sections, available at lower prices, each covering closely related subject-matter divisions. The full annual index goes to all, thus still preserving to sectional subscribers one of the most important long-range features of the whole service wherever there is access to libraries—which, naturally, will want to subscribe to BIOLOGICAL ABSTRACTS complete. Thus, without destroying its integrity, the publication is made available to the different groups of biologists in a form that provides many of the advantages they would enjoy if each society had its own abstracting journal.

The following sections will be available in 1939:

I. *Abstracts of General Biology* to include General Biology, Biography-History, Bibliography, Evolution, Cytology, Genetics, Biometry, and Ecology; price $4.

II. *Abstracts of Experimental Animal Biology* to include Animal Physiology, Nutrition, Pharmacology, Pathology, Anatomy, Embryology, and Animal Production; $9.

III. *Abstracts of Microbiology and Parasitology* to include Immunology, Bacteriology, Viruses, Parasitology, Protozoology, and Helminthology; $5.

IV. *Abstracts of Plant Sciences* to include Phytopathology (with plant viruses), Plant Physiology, Plant Anatomy, Paleobotany, Systematic Botany, Agronomy, Horticulture, Forestry, Pharmacognosy, and Pharmaceutical Botany; $6.

V. *Abstracts of Animal Sciences* to include Paleozoology, Parasitology, Protozoology, and Helminthology, Systematic Zoology, and Economic Entomology; $6.

The price of the complete volume 13, for 1939, will be $25 to individual subscribers and institutions alike.

It was with regret that the trustees, after careful study, found that, in the absence of subsidy, personal subscriptions cannot be offered at a reduction, as heretofore. At the uniform prices set, it will require something like 1250 subscriptions to the complete edition and four times as many to the sections to insure publication without deficit. The libraries will doubtless take care of the former. It is expected that the usefulness and economy of the section

plan will insure the latter. As in the past, members of societies contributing $2 or more per person to BIOLOGICAL ABTRACTS will be entitled to (a) deduct this from subscription price or (b) receive the complete index.

Plant pathologists will be interested particularly in Section IV, ABSTRACTS OF PLANT SCIENCE. Subscription blanks will be sent shortly to members of all societies represented in the Union of American Biological Societies. It would be appreciated if subscriptions could be sent in at once to BIOLOGICAL ABSTRACTS, c/o University of Pennsylvania, Philadelphia, Pa., in order that the management may receive an early indication of the number to be provided for.

It is necessary to add that the Board sees no other plan possible for the continued publication of BIOLOGICAL ABSTRACTS, since it should and must be fully self-supporting hereafter. This will be possible if the volume of sectional subscriptions promptly reaches the expected level.

The American Phytopathological Society
Committee on Biological Abstracts.